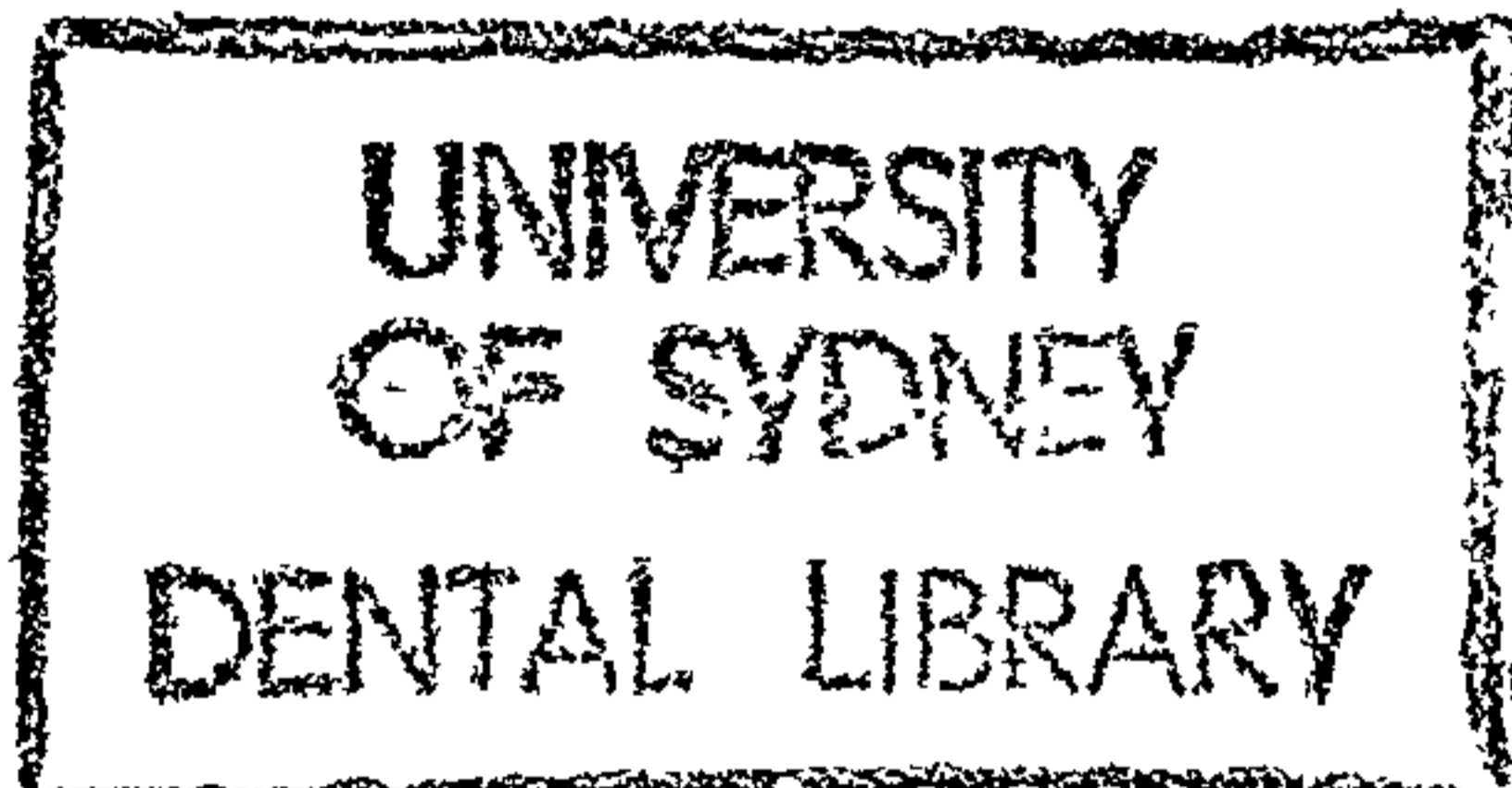


THE  
EFFECT OF TETRACYCLINE ON DEVELOPING  
TEETH

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## D E D I C A T I O N

To my dear wife Helen, and to my children

Hector, Hernan, Susan and Hubert

whose many sacrifices, love and

understanding served as a guiding

force towards this achievement.....

To the unsung Heroine who kept me alive, and

on the move....to carry on when the sailings

go rough --- She, whose inspiration helped

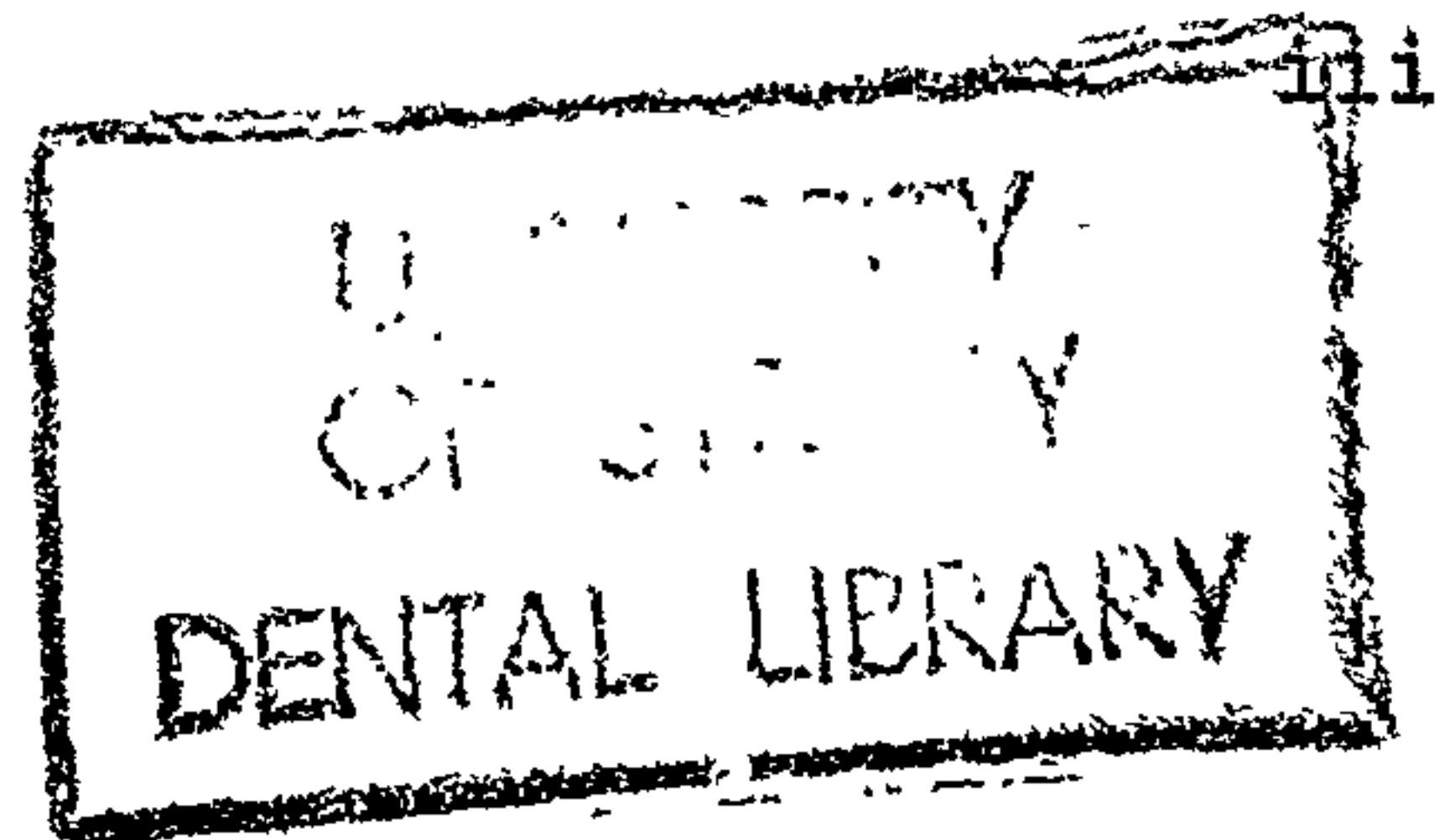
me surmount the seemingly unsurmountable

odds in the thick foliage of both academic

and non-academic endeavors .....

Mother Nature!

PREFACE



The emergence of challenge on the road to contemporary dentistry and medicine is nothing new. It stems from the uncertainty which is expected of scientists of both professions who should be conscious of the fact that science, like human life, is an operation that moves forward. Like life, it confronts new menaces, and meets new problems as it fights its way towards maturity and recognition. The dentists and the physicians, men of science as they are, must stand ready to overcome these menaces. They must not....they cannot, shelve or shirk from the repertory of problems at hand.

One of the challenges that pose a problem, is disease therapy. Clinical and laboratory researchers are pooling their resources to accept this challenge of the times. They are indeed waging a gigantic war towards more naturalistic experimental criterion in the treatment of diseases. They pry open and probe into the enigmas of genetics and the secrets of ecology. In the process, they decipher nature as well as the human organism. They want to be able to anticipate the biological destiny of man and to make therapeutics a subtle diagnostic key with which to unlock the last door towards the maintenance of health, prevention of disease and preservation of life itself.

This thesis deals with the antibiotic therapy. Recent years have seen the introduction of so many antibiotics which showed

considerable therapeutic promise. One of these is tetracycline discovered in 1948. While this drug is one of the most potent antibiotics against many organisms, it has also its limitations. A variety of side effects may ensue from its use. In this thesis, these untoward reactions which affect the developing teeth, will be discussed. Such presentation will be based upon, and supported by the weight of evidences gathered from clinical and laboratory investigations.

For a better understanding and appreciation of the possible effect of tetracycline on developing teeth and the possible mechanism involved, its historical background, chemistry and mechanism of action be reviewed. Different views on growth and development, as well as the chronology of appositional growth of teeth have been presented. Due to selective fixation property, as well as staining in bone and teeth which seems to be characteristic of the tetracycline fluorophore, the possible mechanisms of such fixation on these calcifying tissues are jointly projected. Both animal and human studies relative to discolouration and/or hypoplasia are evaluated. A short discussion on the effect of tetracycline on other tissues is also included. Case reports and retrospective studies on children in New South Wales are also included.

It is hoped in this review of the literature, the case reports and retrospective studies will contribute towards the collation into a rational picture of the fragmentary information concerning the effect of tetracycline on developing teeth. Much of the information

is now widely scattered in the scientific literature journals and more research to be undertaken and published.

The author is very much indebted to Professor N.D. Martin, M.D.S., F.A.C.D., F.I.C.D., F.A.P.H.A., A.I.A.O.P., Head of the Department of Preventive Dentistry, Faculty of Dentistry, University of Sydney, in the preparation of this thesis. His able guidance, his unselfishness in making available his data and clinical photographic records for my use, his kind understanding, patience and constant encouragement were indeed very instrumental in the realisation of this work. I wish also to express my sincere appreciation and gratitude to Mr. Peter Barnard, Senior Lecturer of the department, to Miss Barbara McIntosh and all the members of the staff for their kind cooperation and help.

Last, but not least, I am most grateful to my benefactors.... the University of the Philippines and the governments of the Republic of the Philippines and Australia, without whose support, this academic pursuit would not have been possible.

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## I. INTRODUCTION

The great therapeutic value of tetracycline compounds has been soundly established over a long period of clinical usage. These compounds have a wide range of effectiveness combined with low toxicity and minor side effects. The wide popularity enjoyed by the tetracyclines in common with other antibiotics, however, led to their indiscriminate use. Side effects associated with their administration which until recently, have been confined to allergic reactions, sensitisation of patients, imbalance of normal bacterial flora and emergence of resistant bacterial strains, began to increase and unwanted and sometimes serious results began to create problems for both the medical and dental professions.

Recently, tetracyclines as a group gained the particular attention and interest of the dental profession. Recent observations associating permanent discolouration of teeth ranging from yellow to grey-brown to blue-brown have been reported by various investigators. Hypoplastic and hypocalcified enamel have been reported in literature to accompany this staining of the teeth. There seems to be a consensus of opinion that these conditions followed systematic tetracycline therapy administered during the active tooth formation. The fact that tetracycline crosses the placenta to affect the teeth of foetus when administered to the mother in the later half of pregnancy, has been reported by many investigators.

There is indeed a vast wealth of information about tetracycline scattered widely in scientific publications. Clinical and laboratory

investigations have been undertaken and are still being undertaken and controversies resulting from these investigations continue to appear in scientific literature. The validity of the different hypotheses proposed has yet to be established by further experiments.

This thesis, therefore, aims to integrate these facts in order that a better understanding and appreciation of the possible mechanism of the effects of tetracycline on developing teeth can be established. While there is relatively little that can be done to alter the course of events after development of such anomalies has begun, it may be possible for dentists to minimise or prevent occurrence of anomalies through knowledge and understanding of the etiology, the effect of different tetracycline compounds, pathology and the chronology of tooth development.

## II. THE TETRACYCLINES

Historical Background:- In 1948, Duggar<sup>34</sup> discovered an antibiotic produced by the fungus, Streptomyces aureofaciens. The trade name given was Aureomycin and is officially known as chlortetracycline. Two years later, Terramycin was isolated by Finlay<sup>37</sup> from Streptomyces remosus, and became known as oxytetracycline. A process of synthesising tetracycline by catalytic hydrogenation of the chlorine radical of chlortetracycline was patented by Conover in 1955.<sup>25</sup> McCormick<sup>72</sup> isolated demethylchlortetracycline in 1957. It was produced by a mutant of Duggar's original strain. It was not until two years later, however, that this was first introduced into clinical use. To date, five homologues of the tetracycline family have been introduced for medical use.<sup>31</sup> These are: (a) Tetracycline (TC); (b) Oxytetracycline (OTC); (c) Chlortetracycline (CTC); (d) Demethylchlortetracycline (DMTC) and (e) N-pyrrolidinomethylchlortetracycline (RTC). These are known in the market under various trade names (Table I).<sup>48</sup>

Chemistry of Tetracycline:- The tetracyclines are obtained by fermentation from Streptomyces species and their chemical identities have been established by degradation studies.<sup>31</sup> The important members of the group are derivatives of an octahydronaphthacene, a hydrocarbon that is made up of a system of four fused rings. It is from this system that the group name is obtained. The antibiotic spectra and chemical properties of these compounds are very similar, but not

Table I. Trade names of the five compounds of tetracycline group.  
(West and Todd)<sup>123</sup>

GENERIC NAMES	TRADE NAMES
Tetracycline (TC)	Achromycin, Panmycin, Polycycline, Tetracyn, Staclin, Cyclomycin, Tetradecin, Agromeacina, Sanclomycin, Purocyclina, Tetrabon, Criseociclina, Ambramicia
Oxytetracycline (OTC)	Terramycin, Biostate, Ryomycin
Chlortetracycline (CTC)	Aureomycin, Biomycin, Biomitsin, Aureocina, Acromize, Chrysomykeil
Demethylchlortetracycline (DMTC)	Declomycin
N-(pyrolidinomethyl)-chlortetracycline (RTC)	Tetracycline, Synthetrin, Velacycline, Rolitetracycline, Rerverin

*amphibiotic*  
?  
identical (Fig. 1).<sup>31</sup> The tetracyclines are amphoteric compounds forming salts with either acids or bases.

Tetracyclines have an affinity for polyvalent cations such as calcium, and tetracycline-calcium complexes occur. Under the influence of strong light, the tetracyclines are unstable, and darken in colour. Tetracycline fluoresces a golden yellow colour in ultraviolet light. This property is used when studying the distribution of tetracycline in the tissues.

Mechanism of Action:- Stable chelate complexes are formed by tetracycline with many metals including calcium, magnesium and iron.<sup>31</sup> These antibiotics have strong binding properties with metals. It was suggested that their antibacterial properties may be due to an ability to remove essential metallic ions as chelated compounds. It appears, however, that chelation does not play a big role in the action of tetracycline, but may facilitate transport of the compounds at their site of action. Some investigators concluded that the antibiotic acts principally by interference with the protein synthesis. Many observed effects of tetracyclines are probably remote results of more fundamental disturbances of cellular metabolic pathways. Wood and Archer,<sup>129</sup> stated that the bacteriostatic action of tetracyclines inhibits cytoplasmic metabolism which is essential to bacterial growth.

It is known that tetracycline can form complexes with calcium at physiological pH ranges, and two theories have been proposed.<sup>67</sup> One theory suggests that the deposited material is a tetracycline-

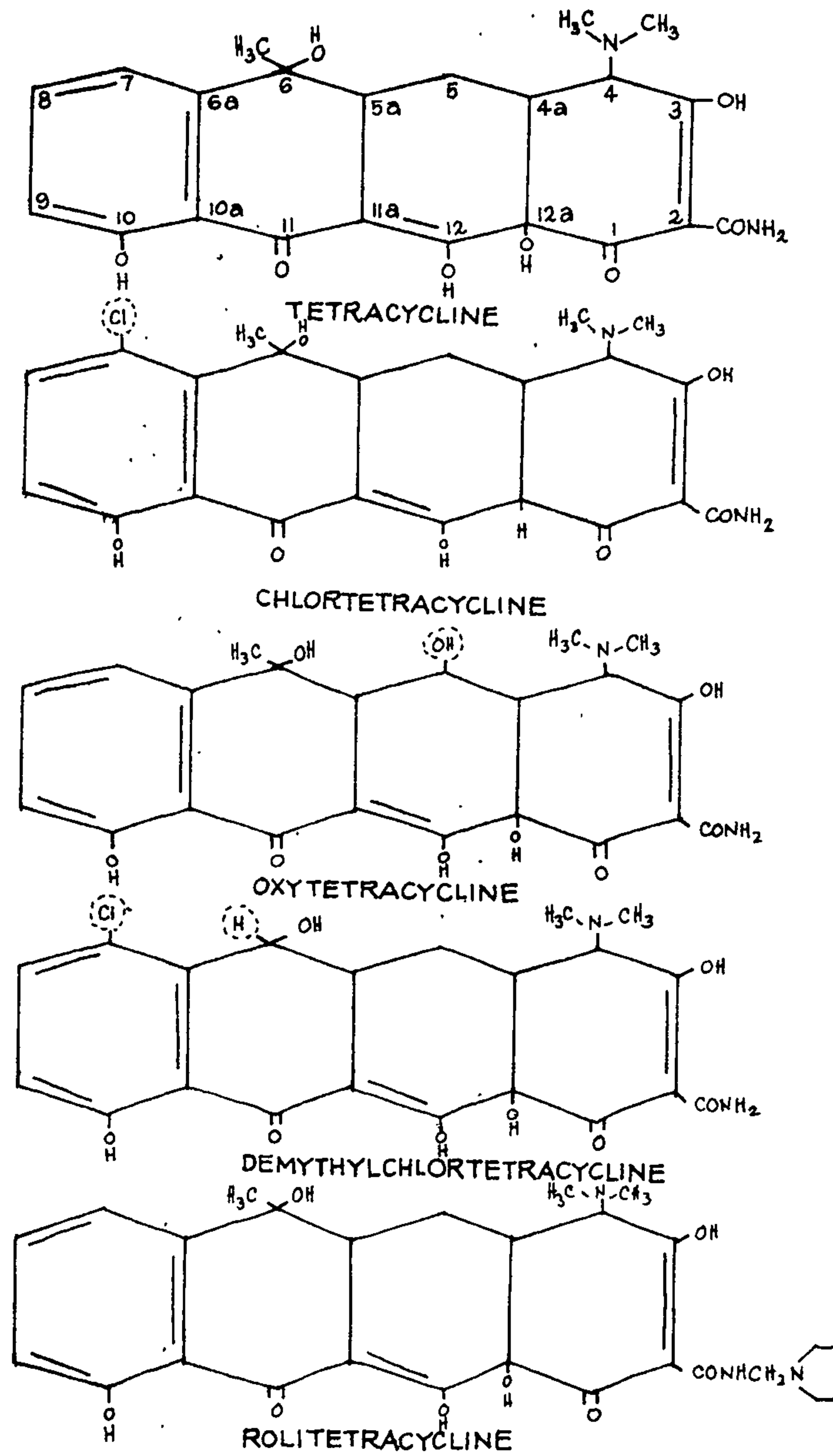


FIG. 1  
Chemical Formula of the Tetracyclines

Fig. I

Chemical Formula of the Tetracyclines.  
(West and Todd)<sup>123</sup>

calcium-orthophosphate complex related to the inorganic surface crystals of bone (Milch,<sup>74</sup> Malek<sup>68</sup>). Another theory by the same investigators implied that tetracycline is attached to the organic bone matrix and tumor tissues by certain peptides.

The members of the tetracycline family are given for specific infections and categorised accordingly (Table II). They occur in various forms and are given daily in certain specified dosage ranges.

Table II. Category dosage range and form of the tetracycline compounds.  
(West and Todd)<sup>123</sup>

NAMES OF DRUGS	CATEGORY	USUAL DOSAGE & RANGE	OCCURENCE
Tetracycline	Antibacterial Antirickett- sial	500 mg 4 times daily 1-2 Gm. daily	Tablets, Capsules Oral suspensions Injection Ophthalmic solu- tion.
Chlortetra- cycline	Antibiotic Antiprotozoan	250 mg. 4 times daily	Capsules Ophthalmic Injection
Oxytetra- cycline	Antibiotic Antiprotozoan	250 mg 4 times daily	Capsules Suspension Ophthalmic
Demethylchlor- tetracycline	Antibiotic	150 mg. 4 times daily or 300mg 2 times daily	Capsules Syrup Suspension
Rolitetracycline	Recommended where the oral dosage forms are not suitable. Introduced for use by intra- muscular and intravenous injections.		

### III. REVIEW OF GROWTH AND DEVELOPMENT OF TEETH

Staining and possible hypoplasia resulting from tetracycline administration, have been observed in calcifying tissues. In the tooth, interest has been directed primarily to the effect in enamel and dentine. A review of the tooth structure development and the chronology of appositional growth will give a better appreciation and understanding of this problem. It will also play an important role in the interpretation of clinical findings, and would be of value in arriving at a correct diagnosis of developmental anomalies related to the effect of tetracycline on the developing teeth.

Development of teeth in the human embryo begins during the sixth week of foetal life. This is manifested by the appearance of an ectodermal thickening, the dental lamina, in the epithelial covering of the primitive ridge areas. The dental lamina is formed as a result of localised, rapid proliferation of epithelial cells which thickens progressively extending into the underlying mesenchyme. Soon after formation, another thickening and downward proliferation of oral epithelium occurs. This is labial and buccal to the dental lamina and separates it from the future lips and cheeks.<sup>23</sup>

During the eighth week of pregnancy, the dental lamina on each side of the jaw shows five discrete round bud-like enlargements. These become the primordia of the primary teeth. These tooth germs consist of an ectodermal part and the mesodermal part.<sup>23</sup> Brauer and

coworkers,<sup>18</sup> classified these specialized structures into three groups, namely: (a) the propriodontal structures which are peculiar to the tooth enamel and dentine; (b) the pulp or endodontal structure... the formative organs of the dentine; and (c) the periodontal structures composed of the cementum, alveolar bone, periodontal membrane and the gingiva.

The dental lamina also initiates the successors of the primary teeth. These originate from the extension of the dental lamina<sup>of the</sup> tooth lingual/<sup>and distal</sup> to the corresponding primary tooth germs. This varies from the fifth month in utero for the central incisor to ten months of age for the second bicuspid. The dental lamina extends beyond the second primary molar tooth germs to form the first, second and third permanent molars. This occurs from the fourth month of the intra-uterine life to the first year after birth with regards to primary dentition, and from four to five years of life in the second and third secondary molars.

A. Amelogenesis:- The ameloblast is directly responsible for the development of the enamel. According to their function, the life span of the cells of the inner dental epithelium can be divided into six stages:<sup>103</sup> (a) Morphogenic; (b) Organising; (c) Formative; (d) Maturation; (e) Protective; and (f) Desmolytic. However, the emphasis in this thesis will be only on the formative and maturation stages. These are the stages more closely concerned with possible developmental anomalies related to tetracycline administration.

Based on the ultimate structure and composition, two processes

are involved in enamel development; (a) the formative stage or organic matrix formation, and (b) the mineralisation or maturation stage.

1. Formative Stage:- The presence of dentine seems to be necessary to initiate enamel matrix formation, so that the ameloblasts enter their formative stage after the first layer of dentine has been produced. During the formation of enamel matrix, the ameloblasts retain approximately the same length and arrangement. The first enamel matrix is deposited extracellularly on a thin layer, the dentino-enamel membrane, along the dentino-enamel junction (Orban and Sicher,<sup>88</sup> Quigley,<sup>93</sup> and Fearnhead<sup>36</sup>). This membrane is continuous with the interprismatic substance that forms subsequently. This would account for the fact that the distal ends of the enamel rods are not in direct contact with the dentine. Matrix is then deposited between the distal ends of the ameloblasts, completely surrounding the ends of the cells, and delineating the Tomes' process. During this time the Tomes' processes begin to form, the terminal bars appear at the distal ends of the ameloblasts. These bars separate the process from the cell proper. A "filling in" of the distal ends of the Tomes' process with matrix material to form segments of enamel rods follows (Scott and Nysten,<sup>99</sup>). Such transformation of the Tomes' process into matrix is repeated over and over again until the entire thickness of the enamel is formed (Fig. 2). This rhythmic deposition of four microns daily, results in primary segmentation and is considered to be the basis of cross striations seen in mature rods.

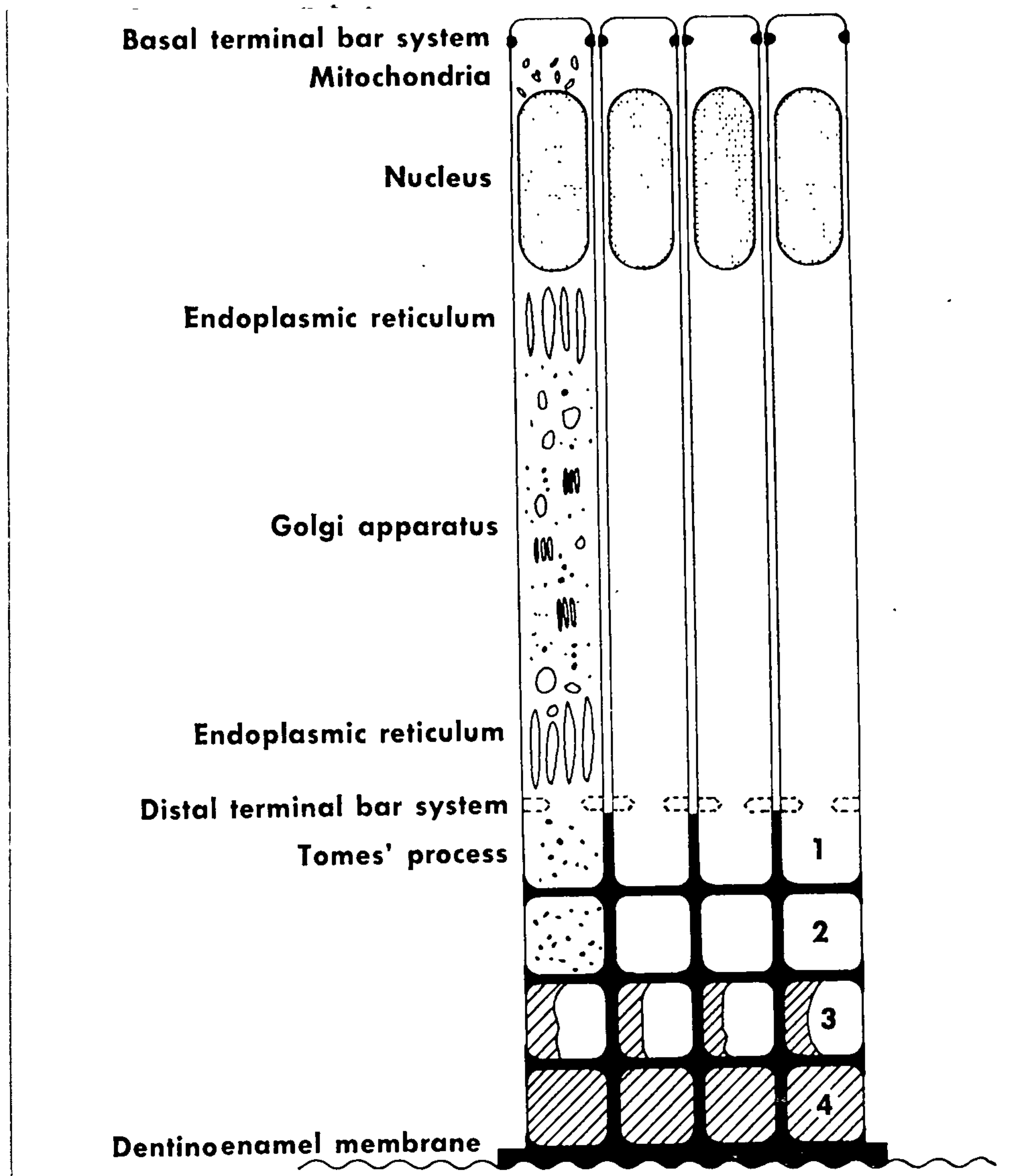


Fig. 2 Diagrammatic illustration of enamel matrix formation.

The ameloblast and enamel rod are shown in linear arrangement, although they are always at an angle to each other. (Sicher)<sup>103</sup>

2. Mineralisation:- After most of the thickness of the enamel has been formed in the occlusal or incisal areas, enamel maturation occurs. At this stage, enamel matrix formation is still progressing at the cervical parts of the crowns. Mineralisation of the matrix takes place in two stages, but the interval between these stages appears to be very small (Crabb,<sup>27</sup>). In the first stage, an immediate partial mineralisation occurs in the matrix segments and in the interprismatic substance as they are laid down. In 1943, Weinman,<sup>121</sup> indicated that initial influx may account for the 25 to 30 per cent of the eventual total mineral content. Frank and Sognnaes,<sup>39</sup> have shown that this first mineral is hydroxyapatite crystallites. This was arrived at through the use of X-ray diffraction and electron microscopy.

The second stage, or maturation, is characterised by the gradual completion of mineralisation which starts from the height of the crown and progresses cervically (Fig. 3).<sup>103</sup> Allan<sup>2</sup> and Crabb<sup>27</sup> stated that maturation seems to begin at the dentinal ends of the rods. There is therefore, an integration of two processes...each rod matures from the depth of the surface, and the sequence in maturing crowns begin from the tips of the cusps or incisal edge and proceeds toward the cervical line. Maturation goes on in the inner, first formed matrix, at the same time as initial mineralisation is taking place in the outer, recently formed matrix. This results from the fact that the maturation process begins before the matrix has reached its full thickness. The basic pattern is at first parallel to the dentinoenamel junction, then to the outer enamel surfaces. Hence, the incisal and

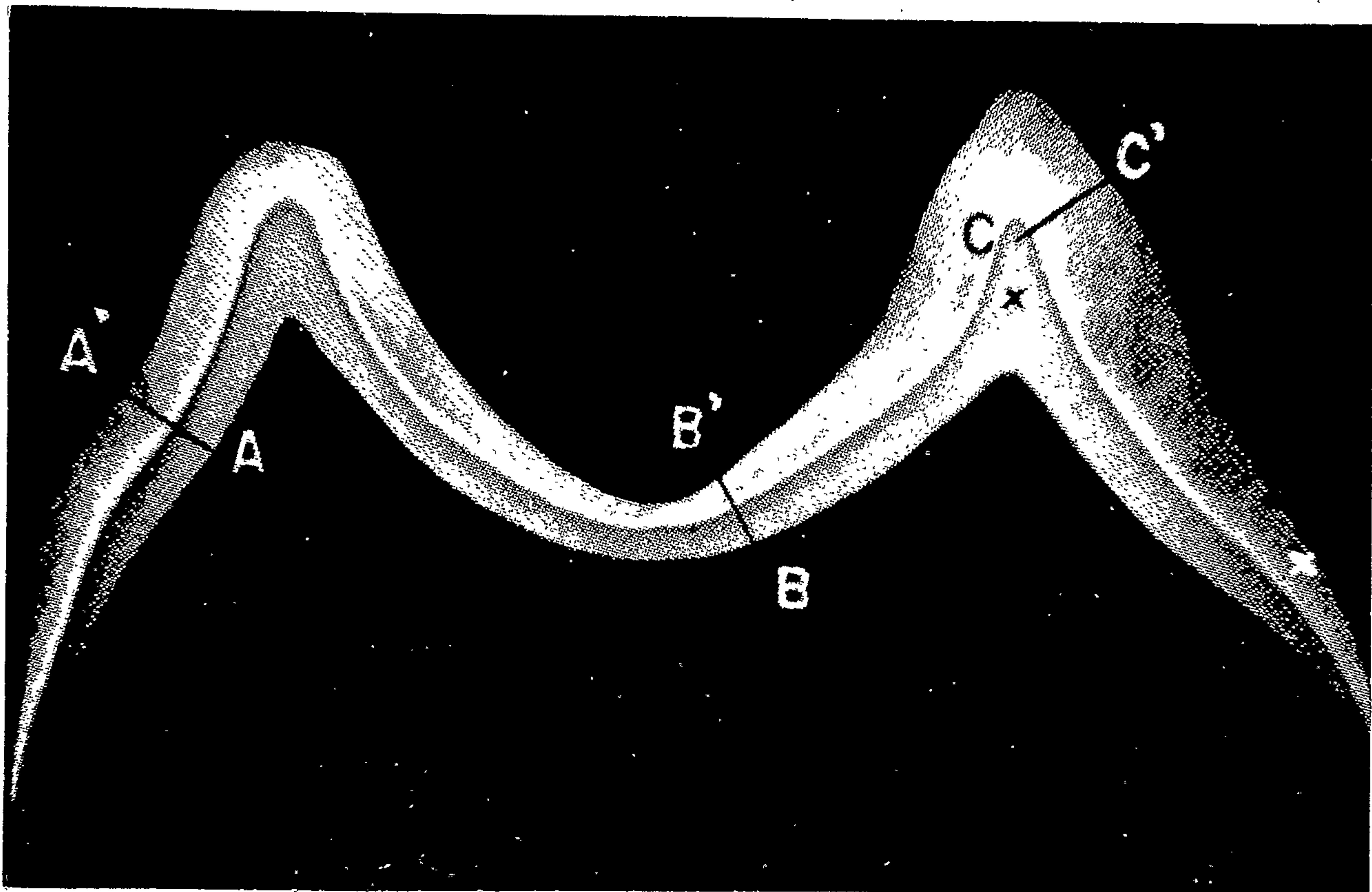


Fig. 3 Microradiograph of a ground section through a developing deciduous molar. From the gradation in radiopacity, maturation can be seen to progress from the dentinoenamel junction toward the enamel surface. Mineralization is more advanced occlusally than in the cervical region. The lines A, B, and C indicate planes in which actual microdensitometric tracings were made. (Sicher)<sup>103</sup>



occlusal regions reach maturity ahead of the cervical region (Fig. 4). It was suggested by Frank and Sognaes,<sup>39</sup> Scott and Nylén,<sup>99</sup> and Rohnholm,<sup>95,96</sup> that maturation at the ultrastructural level, is characterised by growth and eventual fusion of the crystals seen in primary phase. The elongated hexagonal prism cross sections may be attributed to the fact that these original ribbon-like crystals increase more rapidly in length as compared to their width (Nylén,<sup>85</sup> Travis and Glimcher<sup>114</sup>). In 1952, Sognaes and coworkers,<sup>104</sup> stated that the fibrils of organic matrix gradually become thinned and more widely spaced to make room for the growing crystals. There was speculation that some of the organic fibrils actually become imbedded in the crystals (Scott,<sup>99</sup> Rohnholm<sup>95,96</sup>).

Brauer and co-authors,<sup>18</sup> considered that while the basic formative plan at each growth centre is the same for all classes of teeth, the final incremental pattern differs for the different classes. This is determined by the number and position of their individual growth centres. When the cones or adjacent growth centres with formative cells enter into the sphere of influence of one another, a fusion of the products of cellular activity occurs so that the succeeding incremental layers take on the gross outline of the dentinoenamel junction itself (Figs. 5 and 6).<sup>18</sup>

In connection with the nucleation of enamel, hydroxyproline has been detected in both matrix (Piez<sup>90</sup>), and the enamel of unerupted teeth (Burrows<sup>20</sup>). This led to speculation that collagen may be present in an early stage of enamel development and could be the

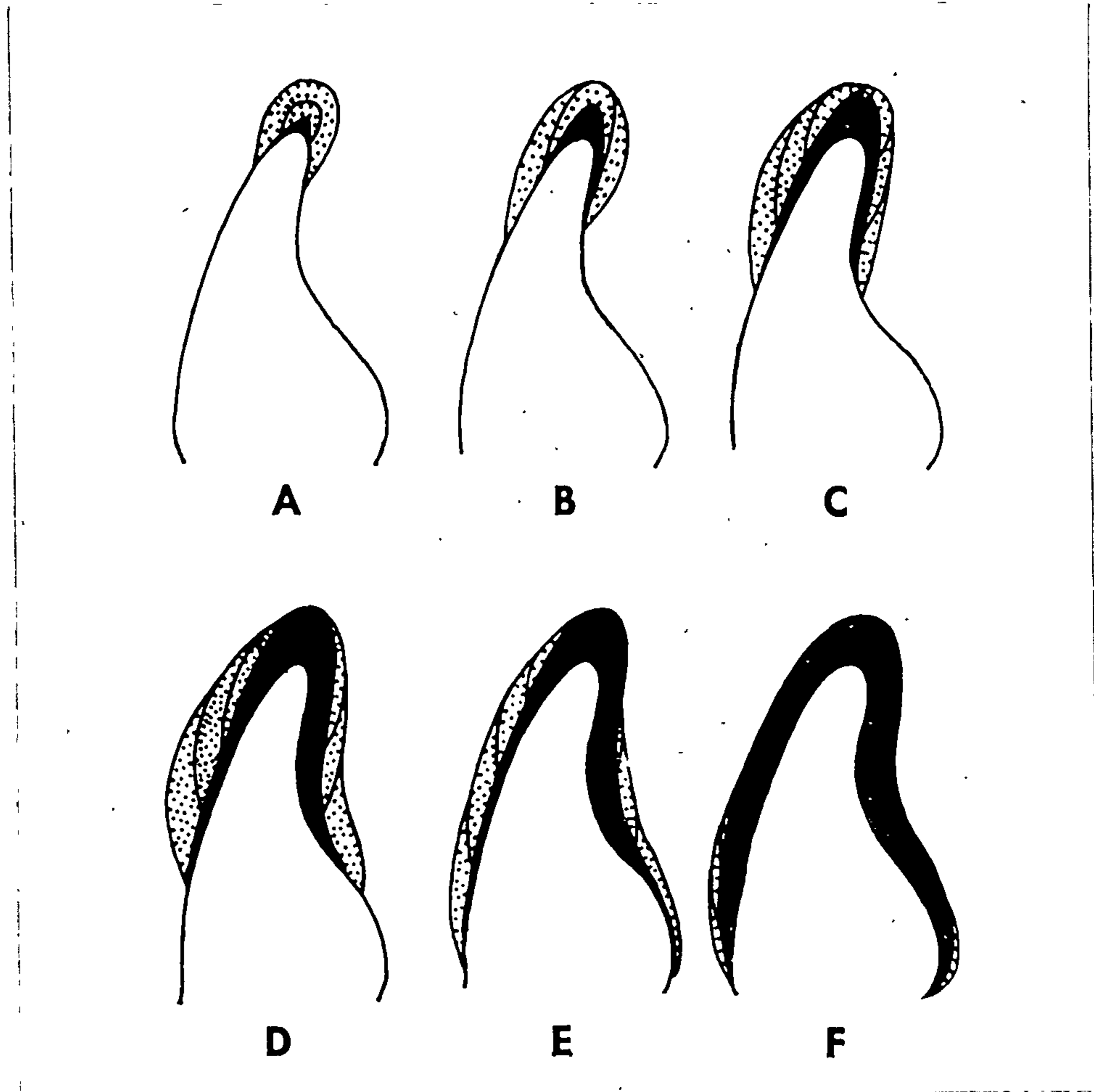


Fig. 4 Diagram showing the pattern of mineralisation of an incisor tooth. The stippled zones represent consecutive layers of partly mineralized enamel matrix. The black areas indicate the advance of final mineralisation during maturation.

(Sicher)<sup>103</sup>

nucleating agent of enamel. If this is so, it would be very sparsely distributed compared with the collagen of bone and dentine. Calculations based on hydroxyproline content of total protein from developing enamel suggest that collagen cannot form more than 5 per cent of the total protein of an early stage of enamel development. With collagen as nucleating agent of enamel, it would be expected to give rise to a comparatively small number of crystallites, relative to the volume of tissues. The crystallites therefore, have more room to grow in the presumably favorable environment provided by the main protein component of developing enamel (Glimcher,<sup>43</sup> and Piez<sup>90</sup>). The crystals grow to fill all the space available and consequently reach a much larger size than in bone and dentine. The crystals of enamel are particularly large when compared with other specimens of hydroxyapatite in biological systems and even those which can readily be produced in vitro. The production of large crystals seems to be a special characteristic of enamel. This could possibly account for the difference in uptake of tetracycline, retention of the fluorophore and staining potential of enamel and dentine.

The final product of the ameloblast is the enamel cuticle.

This is the thin organic membrane that covers the entire enamel surface.

B. Dentinogenesis:- This is a two-phase sequence process:<sup>103</sup>

(a) the elaboration of an uncalcified organic matrix - the preentine and (b) mineralisation. The second phase does not begin until a fairly wide band of preentine has been laid down. The width of the preentine therefore, remains relatively constant until the matrix

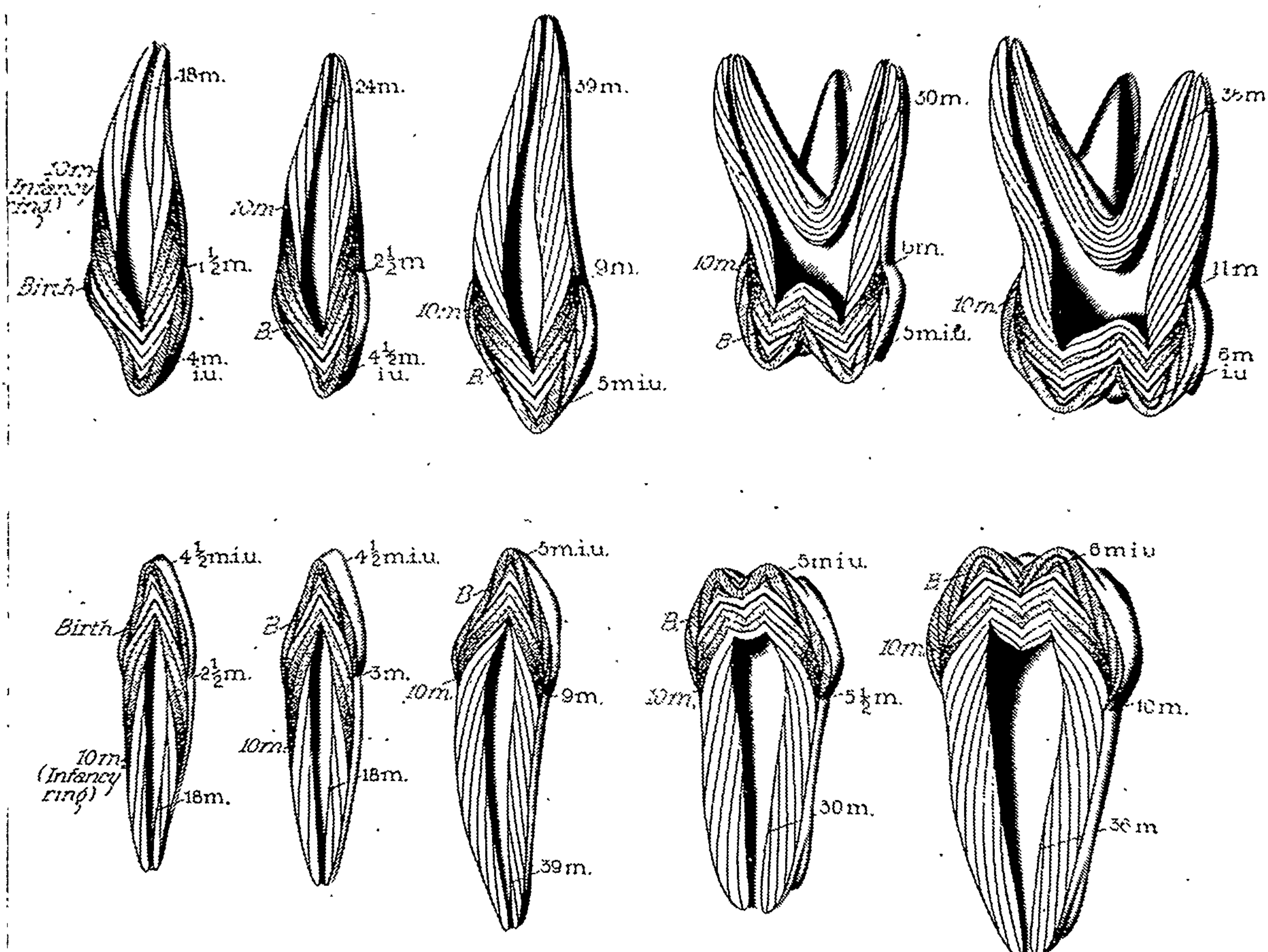


Fig. 5 Diagrammatic representation of the calcification pattern superposed on the incremental pattern of the primary teeth in labiolingual sections. On the right of each tooth is indicated the approximate age at the beginning of teeth formation, at the completion of the crown, and at the completion of the root. Each increment represents approximately 3 months of tooth development. On the left of each tooth is indicated the level of the neonatal ring (birth) and the infancy ring (ten months). The infancy period (birth to 10 mos.), is stippled. In this figure m signifies months and m.i.u., months in utero. (Massler et al) 71A

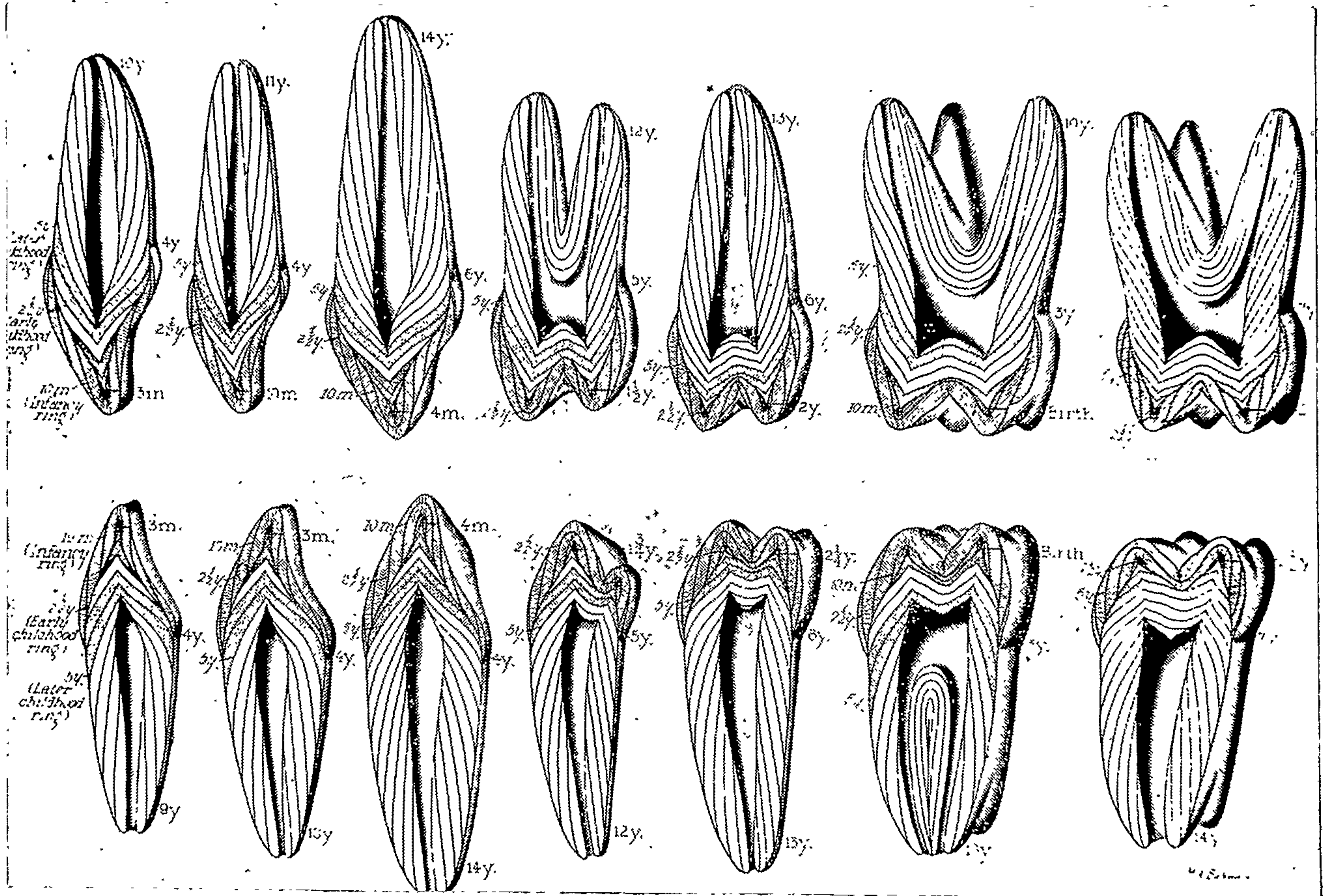


Fig. 6 Diagrammatic representation of the calcification pattern superposed on the incremental pattern of the permanent teeth in labiolingual sections. Each increment represents approximately 1 year of tooth development. On the right of each tooth is indicated the approximate age at the beginning of each tooth formation, at the completion of the crown, and at the completion of the root. On the left of each tooth is indicated the level of neonatal ring (birth), the infancy ring (10 mos.), the early childhood ring ( $\frac{1}{2}$  yrs.), and the later childhood ring (5 yrs.). The infancy and later childhood periods are stippled in the dentin. In this figure m signifies months and y, years. (Massler et al)<sup>71A</sup>

is completed. The formation and calcification of dentine proceed inward from the tips of the cusps or incisal edges, by rhythmic apposition of conic layers...one within the other.

1. Predentine Formation:- The appearance of bundles of fibrils between differentiating odontoblasts, marks the first sign of predentine formation. The cells assume a funnel-shaped configuration near the basement membrane, and the fibrils diverge in a fan-like arrangement to form the Korff's fibres. The origin and role of these fibres in dentinogenesis is still not clear. Some investigators (Orban,<sup>87</sup> and Bevelander<sup>10</sup>), suggested a precollagenous nature because of its argyrophilic reaction. More recent electron microscopic investigations, however (Nylen and Scott<sup>83</sup>), revealed that at their first appearance, the fibrils already demonstrate all the structural characteristics of collagen itself.

The Korff's fibres are a major constituent of the first formed matrix. The mantle dentine comprising the narrow layer near the basement membrane is made up of these Korff's fibres and smaller collagen fibrils. The latter fibrils which form a network, predominate throughout all of the succeeding circumpulpal predentine layers. At this stage, the Korff's fibres, now compact bundles of parallel fibrils, become a minor component. Electron microscopy studies of Watson and Avery,<sup>119</sup> Nylen and Scott,<sup>83</sup> Frank and Nalbandian,<sup>40</sup> have shown that the smaller collagen fibrils in the predentine are formed in the immediate vicinity of the distal ends of the odontoblast. As to the origin of the collagen fibrils in the predentine, the assumption

has been made that these fibrils are produced in the subodontoblastic region from an afibrillar substance, presumably the product of odontoblast.<sup>113</sup>

Takuma<sup>112</sup> said that the presence and the significance of Korff's fibres is still a matter of speculation among electron microscopist. These fibres had been identified by previous investigators and more recently were found in human teeth, both in the developing<sup>82</sup> and mature<sup>52,53</sup> stages. Noble, Carmichael and Rankine,<sup>82</sup> also suggested that Korff's fibres do not play an important part in predentine matrix formation because they were seen only at a later developmental stage aggregated in small amounts between the fully developed odontoblasts.

2. Mineralisation:- Takuma,<sup>111</sup> proposed that mineralisation of the layers closest to the dentino-enamel junction begins in small islands after several microns of predentine have been laid down. These subsequently fuse and form a continuous calcified layer. Mineralisation usually advances pulpward as a linear front roughly paralleling the odontoblastic layer, with further predentine formation. Advancing mineralisation sometimes occurs in globular areas that subsequently fuse. Occasionally, both globular and linear calcification are seen in combination. Takuma described the dentine crystals as being needle, plate-like and granule shaped in the developmental stage. Watson and Avery,<sup>119</sup> Johansen and Parks,<sup>52,53</sup> and Johansen,<sup>54</sup> described dentine crystalites as thin plates, and thinner more dense profiles are interpreted as an edge-on view of some crystals. This view has been positively confirmed by Johansen and Parks,<sup>52</sup> and Johansen<sup>54</sup>

through the stereoscopic analysis of crystals obtained from mature human dentine.

From the beginning, the apatite crystals form and grow in relation to the collagen fibrils (Takuma).<sup>112</sup> These changes commence in a surface layer of the predentine which has developed to a certain thickness and forms small clusters of apatite crystals. In more advanced stages, the clusters develop into irregularly shaped islands which later fuse to form layers of dentine. It is at this stage that an especially heavy accumulation of crystals occurs on the inner walls of the dentinal tubules, thereby increasing the width of this area and resulting in the formation of the peritubular matrix. This is easily distinguished from the intertubular matrix because of its more highly mineralised nature. As the clusters of apatite appear in the predentine, the separating membrane becomes vague and disappears, and enamel matrix starts to form directly on the surface of the dentine formed through the fusion of the islands. Thickening of the dentine layer takes place through additions of the dentino-predentine junction. An acute narrowing of the tubules of the predentine and of the odontoblastic processes occurs at the dentino-predentine junction, as the peritubular matrix forms at a rate similar to that of the intertubular matrix during appositional dentine growth.

The precise mechanism of crystallites formation has yet to be definitely established. The question of how inorganic crystallites are first formed for those hard tissues with a collagenous matrix is far from being settled. A theory known as the "Epitactic Theory",<sup>81</sup>

has since gained support. This involves the concept of catalysis of the formation of crystal "seeds" by a solid surface which has some of the characteristics of lattice of these crystals. Strong evidence suggests that collagen, either of itself or following activation, or acting in association with other macromolecules, has the necessary characteristics for the formation of the hydroxyapatite crystals (Weidman).<sup>120</sup> Once the seeds of inorganic crystals have formed whatever mechanism operates, they would be expected to increase in size as a result of the physical process of crystal growth. This is of course, provided that sufficiently high concentrations of calcium and phosphate ions are maintained within the mineralising system. The crystal growth continues until almost all of the available space is filled.<sup>35</sup>

C. Chronology of Appositional Growth:- The growth process of dentition is subject to considerable physiological variation. Research workers describing the chronology of the human dentition have presented averages for the various age levels (Legros and Magitot,<sup>63</sup> Mummery,<sup>77</sup> Churchill,<sup>22</sup> Logan and Kronfeld,<sup>65</sup> Schour and Massler,<sup>98</sup> Kraus,<sup>60</sup> Scott and Nylén,<sup>99</sup> Kraus and Jordan<sup>59</sup>).

Kraus,<sup>60</sup> placed the initial calcification of the primary teeth in the range of 14-22 weeks in utero. In a study involving 95 fetuses, he established that the time of initiation is variable. He observed that the primary central incisors developed from a single lobe and not from three centers as formerly believed. Kraus gave the following order of initial calcification of primary teeth: (a) Central incisor,

upper before lower; (b) First molars, upper before lower; (c) Lateral incisor, upper before lower; (d) Cuspids, lower may be slightly earlier; and (e) Second molars, simultaneously.

Brauer and associates<sup>18</sup> stated that the appositional growth of teeth begins at different ages, but in a definite and regular pattern and sequence (Table III). Systemic diseases or disturbances occurring during these different stages influence the nature and appearance of the teeth (Table IV). Brauer's groupings are as follows:

Group I. (Prenatal):-- As a group before birth, the apposition of enamel and dentine of primary teeth ranges from 4-6 months in utero, from central incisor to second molar.

Group II. (Birth to 3 months):-- This group involves the first permanent molar and the permanent anterior teeth except the lateral incisor which does not begin until about 10 months of age.

Group III. ( $1\frac{1}{2}$  - 3 years):-- There is a pause, then the bicuspid, and the second permanent molars begin their formation as a group about these ages.

Group IV. (7 - 10 years):-- Another pause, the third molars commence formation.

As a rule, the upper teeth begin formation slightly earlier than the lower. However, the lower generally erupt before the corresponding upper teeth.

Enamel and dentine formation proceeds both regularly and rhythmically, and the time required for the completion of the crown depends on the length of the crown and rate of tissue formation. Upon

Table III. Chronology of Growth of Human Teeth. (Brauer et al.)<sup>18</sup>

CHRONOLOGY OF GROWTH OF HUMAN TEETH					
	<i>Tooth</i>	<i>Tooth Germ Formation</i>	<i>Enamel and Dentin Apposition Begins</i>	<i>Crown Completed</i>	<i>Root Completed</i>
PRIMARY	Central incisor . . . . .	7 w.i.u.*	4-4½ m.i.u.	1½-2½ mos.	1½ yrs.
	Lateral incisor . . . . .	7 w.i.u.	4½ m.i.u.	2½-3 mos.	1½-2 yrs.
	Cuspid . . . . .	7½ w.i.u.	5 m.i.u.	9 mos.	3¼ yrs.
	First molar . . . . .	8 w.i.u.	5 m.i.u.	5½-6 mos.	2½ yrs.
	Second molar . . . . .	10 w.i.u.	6 m.i.u.	10-11 mos.	3 yrs.
	First molar . . . . .	3½-4 m.i.u. †	Birth	2½-3 yrs.	9-10 yrs.
PERMANENT	Central incisor . . . . .	5-5¼ m.i.u.	3-4 mos.	4-5 yrs.	9-10 yrs.
	Lateral incisor . . . . .	5-5½ m.i.u.	10-12 † 3-4 mos.	4-5 yrs.	10-11 yrs.
	Cuspid . . . . .	5½-6 m.i.u.	4-5 mos.	6-7 yrs.	12-15 yrs.
	First bicuspid . . . . .	Birth	1½-2 yrs.	5-6 yrs.	12-13 yrs.
	Second bicuspid . . . . .	7½-8 mos.	2-2½ yrs.	6-7 yrs.	12-14 yrs.
	Second molar . . . . .	8½-9 mos.	2½-3 yrs.	7-8 yrs.	14-16 yrs.
	Third molar . . . . .	3½-4 yrs.	7-10 yrs.	12-16 yrs.	18-25 yrs.

\* w.i.u. = weeks in utero.

† m.i.u. = months in utero.

‡ When significant differences occur between upper and lower teeth, their chronology is indicated separately.

SOURCE: Schour, I., and M. Massler, 1940.

Table IV. Developmental pattern of the child and its reflection in the teeth. (Brauer et al)<sup>18</sup>

DEVELOPMENTAL PATTERN OF THE CHILD AND ITS REFLECTION IN THE TEETH				
Developmental Periods and Tooth Rings	Age Period	Physiologic and Developmental Characteristics	Teeth†	
			Quality of Calcification and Histologic Characteristics	Distribution of Hypoplastic Defects in the Enamel‡
Prenatal	..	Well-protected parasitic existence	Good calcification	Rare
Neonatal ring	Birth to 2 wks.	Neonatal adjustments; birth trauma; arrest in growth	Dark, distinct arrest line in enamel and dentin	7% (acute hypoplasia of neonatal ring)
Infancy period	2 wks. to about 10 mos.	Period of postnatal adjustment; marked susceptibility to infection, disorders of alimentation, and metabolic disturbances; changes in mode of growth from simple to complex	Generally poor calcification, progressively less homogeneous from third to tenth month; period of poorest calcification and greatest susceptibility to chronic hypoplastic defects, early infancy ring present at sixth month	70% (chronic hypoplasia of the infancy period)
Infancy ring	About 10 mos.	Time of greatest susceptibility to diseases of infancy, "critical" period; temporary depression in growth curve	Sharp arrest line in enamel and dentin demarcating infancy from early childhood period; marks a period of acute susceptibility to hypoplastic defects of the enamel as well as the abrupt termination of chronic hypoplastic defects of infancy	5% (acute hypoplasia of the infancy ring)
Early childhood period	About 10 mos. to 2½ yrs.	More independent existence; improved alimentation and antibody mechanism	Recovery in calcification and cessation of hypoplasia abrupt and complete; calcification better than during infancy but not so good as during prenatal period	Relatively rare

Developmental Periods and Tooth Rings	Age Period	Physiologic and Developmental Characteristics	Teeth†	
			Quality of Calcification and Histologic Characteristics	Distribution of Hypoplastic Defects in the Enamel‡
Early childhood ring	About 2½ yrs.	?	A sharp arrest line in enamel and dentin which demarcates early from later childhood periods; marks a period of acute susceptibility to enamel hypoplasia	5-10% (acute hypoplasia of the early childhood ring)
Later childhood period	About 2½ to 5 yrs.	Most of the exanthematous diseases occur during this period	Calcification generally poor but better than during the infancy period	5-10% (chronic hypoplasia of the later childhood period)
Later childhood ring	About 5 yrs.	?	A sharp arrest line in enamel and dentin which demarcates the later childhood from the grade school periods; marks a period of acute susceptibility to enamel hypoplasia	5% (acute hypoplasia of the later childhood ring)
Grade-school age	About 5 to 10 yrs.	?	Calcification generally good but variable	Relatively rare

SOURCE: Massler, M., I. Schour, and H. G. Poncher. 1941.  
† The levels of teeth affected are given in Figs. 3-12, 3-13, and 3-18.  
‡ Because of the extreme sensitivity of the enamel-forming cells to metabolic disturbances, severe disturbances in calcification are manifested also as deficient enamel formation (enamel hypoplasia), which can be recognized grossly. About 5 per cent of the population suffer from these defects. The figures give the distribution of these defects according to age and type and refer to percentages of the total number of hypoplastic defects observed (see Fig. 3-20).

completion of the enamel formation, the crown of the tooth is finished. The primary teeth takes from 7-14 months for formation of the crown. The completion of the crowns of secondary teeth takes from 3-6 years, due to their greater sizes and slower rate of formation. The first permanent molar, due to its relatively rapid rate of formation, takes the least time to develop. The cuspid, both secondary and primary, take the longest time to develop because of the length of the crown.

Conflicting opinions are still prevalent as far as chronology is concerned. Logan and Kronfeld's figures, however, slightly modified by McCall and Schour, are still generally regarded as a valid measure of prenatal tooth development. Coughlin<sup>26</sup> in his study, does not agree with these figures, but follows more closely those presented by Kraus and Jordan.<sup>59</sup> Coughlin's findings relating to the extent of calcification at birth differ from the modified Logan-Kronfeld figures. His results showed that the mean age of coalescence of the cusps was 32 weeks for the mandibular first molar. Coughlin also observed that complete occlusal calcification was present at 33.7 weeks in the maxillary first molar and 35.4 weeks in mandibular first molar, results almost identical with those of Kraus and Jordan.<sup>59</sup>

Gates<sup>41,42</sup> investigated the applicability of the current eruption times of permanent teeth to the Australian child population. He made use of Barnard's data obtained from a cross-sectional survey of a representative sample of New South Wales school children made in 1954-1955. Of the 5660 children examined from 54 different schools in the

country and Sydney Metropolitan area, 2,753 were girls and 2,907 boys, aged 6-15 years of age. They are all white children residents of N.S.W. No great difference between the sequence of eruption, derived from the average age of eruption in this study, and that of other studies was found (Table V). It appeared that females are about 6 months ahead of males as far as commencement and completion of the secondary dentitions are concerned. It is considered normal for secondary dentition to commence eruption between the ages of 5 years and 6 years 4 months for females, and 5 years and 6 years 9 months for males. The range of completion of the permanent dentition with the exception of the third molar is within 11 years to 14 years 7 months for girls, and 11 years 5 months to 14 years 11 months for boys (Table VI).

While most investigators are in agreement that the histodifferentiation and appositional stages of the primary and permanent dentitions extend from the end of the second month of prenatal life to about the six months after birth, and from birth to about 10 years of age, respectively, these are not conclusive. Many questions still arise and many other investigators are being stimulated to seek answers to these questions. Detailed morphogenesis and morphodifferentiation studies have yet to be done.

Table V. Median Ages of eruption of permanent teeth of New South Wales children. (Brearley, Stragis and Storey)<sup>16</sup>

*Median ages of eruption of permanent teeth of New South Wales children*

Tooth*	Females			Males		
	Median	Confidence interval†		Median	Confidence interval†	
		Lower limit	Upper limit		Lower limit	Upper limit
	Yr.	Yr.	Yr.	Yr.	Yr.	Yr.
Upper :						
1. L .. ..	6.80	6.714	6.892	7.12	7.043	7.205
R .. ..	6.79	6.702	6.882	7.10	7.018	7.181
2. L .. ..	7.75	7.666	7.828	8.15	8.066	8.228
R .. ..	7.77	7.694	7.856	8.15	8.072	8.235
3. L .. ..	10.71	10.60	10.81	11.33	11.23	11.43
R .. ..	10.65	10.55	10.76	11.31	11.21	11.41
4. L .. ..	9.80	9.691	9.903	10.20	10.09	10.31
R .. ..	9.83	9.722	9.929	10.25	10.14	10.36
5. L .. ..	10.66	10.54	10.78	10.95	10.84	11.07
R .. ..	10.66	10.54	10.77	10.98	10.87	11.09
6. L .. ..	6.11	5.909	6.314	6.23	6.077	6.393
R .. ..	6.13	5.940	6.327	6.32	6.160	6.490
7. L .. ..	11.75	11.65	11.86	12.12	12.01	12.22
R .. ..	11.74	11.64	11.84	12.12	12.02	12.23
Lower :						
1. L .. ..	5.94	5.678	6.206	6.21	6.063	6.370
R .. ..	5.96	5.721	6.202	6.16	6.004	6.324
2. L .. ..	6.97	6.867	7.064	7.36	7.280	7.436
R .. ..	6.88	6.782	6.977	7.32	7.236	7.396
3. L .. ..	9.40	9.309	9.490	10.48	10.39	10.58
R .. ..	9.41	9.316	9.497	10.51	10.41	10.60
4. L .. ..	10.12	10.01	10.22	10.79	10.67	10.90
R .. ..	10.30	10.19	10.42	10.82	10.71	10.94
5. L .. ..	11.17	11.05	11.30	11.59	11.47	11.72
R .. ..	11.03	10.91	11.15	11.55	11.43	11.66
6. L .. ..	5.85	5.564	6.159	6.14	5.961	6.323
R .. ..	5.95	5.712	6.206	6.12	5.944	6.304
7. L .. ..	11.25	11.15	11.36	11.63	11.53	11.73
R .. ..	11.30	11.19	11.40	11.69	11.58	11.78

\* Tooth code : Teeth are numbered 1 to 7 corresponding to central to second molar. L and R denote left and right sides of the arches respectively.

† Ninety-five per cent. confidence interval of the median.

Table VI. A comparison between the average sequences of eruption of the permanent teeth, which were observed in this and other studies. Teeth of both arches considered as one. (Brearley, Stragis and Storey)<sup>16</sup>

*A comparison between the average sequences of eruption of the permanent teeth, which were observed in this and other studies. Teeth of both arches considered as one series*

Study	Country	Upper jaw							Lower jaw						
		I <sub>1</sub>	I <sub>2</sub>	C	Pm <sub>1</sub>	Pm <sub>2</sub>	M <sub>1</sub>	M <sub>2</sub>	I <sub>1</sub>	I <sub>2</sub>	C	Pm <sub>1</sub>	Pm <sub>2</sub>	M <sub>1</sub>	M <sub>2</sub>
Females															
Present study	Australia	4	6	11/10	8	10/11	3	14	2/1	5	7	9	12	1/2	13
Carr <sup>(3)</sup> ..	Australia	4	6	11	8	10	2/3	14	1	5	7	9	12	2/3	13
Halikis <sup>(4)</sup> ..	Australia	4	6	10	8	11	3	14	2	5	7	9	13	1	12
Clements <sup>(5)*</sup>	England	4	6	10	8	11	3	13	1	5	7	9	14	2	12
Ainsworth <sup>(6)</sup>	England	4	6	11	7	10	2	14	3	5	8	9	14	1	13
Moak <sup>(7)</sup> ..	S o u t h Africa	4	6	10	8	11	3	14	3	5	7	9	12	1	13
Cattell <sup>(8)</sup> ..	America	4	6	11/12	8	10	3	14	2	5	7	9	11/12	1	13
Hurme <sup>(9)</sup> ..	I n t e r- national	4	6	12	8	10	2	14	3	5	7	9	11	1	13
Males															
Present study	Australia	4	6	11	7	10	3	14	2/1	5	8	9	12	1/2	13
Carr <sup>(3)</sup> ..	Australia	4	6	11	7	10	3	14	1/2	5	8	9	12	1/2	13
Halikis <sup>(4)</sup> ..	Australia	4	6	11	7	9	2	13	3	5	8	10	14	1	12
Clements <sup>(5)*</sup>	England	4	6	11	7	12	2	13	1	5	8	9	14	3	10
Ainsworth <sup>(6)</sup>	England	4	6	11	7	10	2	14	3	5	8	9	12	1	13
Monk <sup>(7)</sup> ..	S o u t h Africa	4	6	11	7	10	2	14	3	5	8	9	12	1	13
Cattell <sup>(8)</sup> ..	America	4	6	12	7	10	3	14	2	5	9	8	11	1	13
Hurme <sup>(9)</sup> ..	I n t e r- national	4	6	12	7	10	2	14	3	5	8	9	11	1	13

\* Right side only.

#### IV. EFFECT OF TETRACYCLINE ON DEVELOPING TEETH

Many investigators have established a correlation between tetracycline administration and the discolouration of primary and permanent teeth, as well as the possible hypoplasia of the enamel. Much information has appeared in the literatures describing the particular affinity and selectivity which tetracycline compounds exhibit for bone and tooth substances. This selective fixation property of tetracycline fluorophore complex in tissue undergoing calcification at the time of the administration of this drug has made possible the use of tetracycline as tracer material in both osseous and dental tissue studies, both in animals and humans.

A. Discolouration:- The yellow colour and the golden yellow fluorescence pattern exhibited by the osseous and dental tissues under ordinary and ultraviolet light respectively, are strongly indicative of tetracycline deposition. Both animal and human studies have produced the various data accumulated so far, in order to unravel the possible role of tetracycline therapy in the production of such staining.

1. Animal Studies:- In 1958, Milch and associates<sup>74</sup> reported tetracycline staining in their animal experiments. Deposition of tetracycline in bones, teeth and tumor tissues were demonstrated and also the specific localisation of the tetracycline oxytetracycline in bones. This study also corroborated the suggestion of these investigators that the drug may be employed

in vivo as an effective histological indicator for newly proliferated bone tissue. They used rats and white rabbits, both males and females. In both studies, intraperitoneal doses of each of the tetracyclines at the level of 50 mg/kg of body weight were administered. Doses from 0.1 mg/kg to 200 mg/kg of body weights were given by subcutaneous, intramuscular, and intravenous injections. In some studies, oral administration was used. It was found that induced fluorescence was apparent in widely distributed tissues, except the brain. This was observed almost instantaneously following intravenous injections, and within 30 minutes of the intraperitoneal administration. It was found to persist for approximately six hours in all tissues and was undetectable grossly in soft tissues 12 to 24 hours later. The flat bones, the long bones and the incisor teeth of the rodents showed persistence of fluorophore for prolonged period of time. A more vivid fluorescence was observed after doses in range of 50 to 200 mg/kg of body weight in comparison to minimal fluorescence with dosage range of 0.2 mg/kg.

Zipkin and coworkers,<sup>132</sup> reported that when rats are given tetracycline during gestation, lactation and preweaning period, the drug has caries-inhibitory effect. They observed yellow fluorescence under ultraviolet light, which according to them is strongly indicative of tetracycline discolouration. Bevelander and associates,<sup>12</sup> giving tetracycline to young rats, found that the tetracycline was clearly seen in the incremental pattern of teeth and bone. They gave 5 mg of tetracycline hydrochloride for each of five successive

days and upon examinations of the teeth following such treatment, they found fluorescent bands in the dentine and enamel of the incisors and molars corresponding to the 5 injections of drug administered.

In 1961, Owen<sup>89</sup> demonstrated crossing of the placental barrier by tetracycline. Normal rats about 19 days pregnant were injected intramuscularly with chlortetracycline and killed 24 hours later. Gross examination of the fetuses showed marked yellow fluorescence. Young female rats dosed heavily with chlortetracycline prior to mating and whose bones fluoresced bright yellow under ultraviolet light, produced fetuses whose bones did not show any fluorescence. In the same experiment, a normal dog, 8 weeks of age was dosed regularly for 6 days a week for a month with 750 mg chlortetracycline. Both the enamel and dentine of the dog exhibited pale yellow colouration in daylight, with intense yellow fluorescence under ultraviolet light.

Boyne and Miller<sup>15</sup> gave alternately at 10 days interval, single intravenous doses consisting of 10 mg/kg of body weight of oxytetracycline and chlortetracycline to dogs whose cuspid teeth were developing. When the ground unstained histological specimen was examined with ultraviolet dark field illumination, a yellow fluorescence pattern was observed within the dentine of the animal given oxytetracycline. With chlortetracycline, a deeper yellow pattern resulted. Variations in characteristics of fluorescent bands made possible chronologic orientation of growth pattern of the developing teeth. In addition, characteristic fluorescent increments were produced in the adjacent alveolar bone. They also reported that this

tetracycline-induced fluorescence was found to have persisted in the teeth of dogs twelve months after administration of a single intravenous dose of tetracycline.

Shira,<sup>100A</sup> reported producing a pale yellow stain in permanent teeth of a normal dog to whom he had given chlortetracycline for six days for a period of one month. He was able to observe intense yellow fluorescence of the teeth under ultraviolet light. However, this was not demonstrable in the teeth of littermates. He found four narrow bands corresponding to the days he did not administer the drug. Applebaum and coworkers<sup>4</sup> made a study to determine the effect of tetracycline administration on the colour of the teeth of rats in the process of development. Gross and histologic studies suggested that the discolouration and the fluorescence exhibited under ultraviolet light, was a result of the deposition of tetracycline. Dosage employed was 12.5 mg. of tetracycline per 20 gm. of food during the entire gestation period. This approximately corresponds to the dosage level 50mg/kg administered to infants and children suffering from cystic fibrosis of the pancreas. This dosage was reduced to 12.5 mg/30 gm. of food, following delivery and lactation. The second group in their experiment was given five times the first group and the third group acted as control group. The result showed that group I had nominal colouration and group II had more, and that more intense colouration resulted from higher dose levels.

Storey<sup>109</sup> in his experiments with 14 day old rats given tetracycline intraperitoneally, for five days and observed for the

following fifty seven days, found that molar teeth were coloured bright yellow and fluoresced under ultraviolet light. Dosage level used was 20 mg/kg per day and the fluorescence was observed from 15 minutes onward. It appeared in growing bones as zones of yellow staining with corresponding zones of yellow photofluorescence under ultraviolet light. Throughout the bones, tetracyclines are deposited uniformly and higher concentrations are observed in areas undergoing calcification. They remain until both matrix and calcium salts are removed during remodelling. It was observed that in the higher dose levels of 200-250 mg/kg/day, yellow and brown pigmentations resulted. Fluorescent microscopy showed initial localisation of tetracycline to margins of calcifying enamel where the crowns were formed and enamel calcification is incomplete. None, however, was discernable where amelogenesis and dentine apposition has commenced.

Lewis<sup>64</sup> mentioned Gron and Johansen as having demonstrated in rats at the higher of the two dosage levels used, two distinct sets of fluorescent lines in dentine corresponding to the four injections given. Cuttita<sup>28</sup> and coworkers, in their effort to obtain specific evidence that tetracycline is deposited in forming teeth and that it is discernable both clinically, as a discolouration, and histologically, using various microscopic techniques, undertook studies in both dogs and rats. Tetracycline was administered in accordance with varying dosage regimens during the course of gestation and after different periods. Clinically, the offspring revealed that the teeth of

dogs were discoloured while the teeth of rats were noticeably discoloured only in the higher dose ranges. Histologic studies viewed by both incandescent and ultraviolet lights indicated to presence of tetracycline. In 1966, Bevelander and Nakahara<sup>14</sup> injected young rats with varying amounts of tetracycline for several successive days. They were examined to ascertain presence of fluorophore and discolouration of developing enamel and dentine. They found that higher doses gave more fluorescence and colouration of enamel and dentine. In young rats of two weeks old, more intense fluorescence and visible pigmentation of enamel and dentine were observed as compared with older rats. They concluded that large doses affect the degree and extent of tetracycline discolouration. Manson<sup>70</sup> in his study of bone activity and growth in animals given 4-5 day human adult dose of tetracycline, found little if any observable macroscopic signs of pigmentation. Label is clearly visible, however, in sections of enamel and dentine when viewed under ultraviolet light.

2. Human Studies:- Discolouration of the teeth by the tetracycline group of antibiotics was first reported by Shwachman and Schuster in 1956<sup>101</sup> in their studies on a group of over 300 infants and children suffering from cystic fibrosis of the pancreas treated with chlortetracycline and oxytetracycline for one year or more. They found that approximately five per cent showed discolouration of the deciduous teeth. Later, Shwachman and coworkers<sup>102</sup> in their studies on 50 children with cystic fibrosis treated with broad

spectrum antibiotics, mainly tetracycline for at least eight years, found that 40 children or 80 per cent had darkly stained teeth. Zegarelli and associates<sup>130</sup> reported that tooth discolouration tended to localise in the middle and cervical thirds of the clinical crowns. These were children with cystic fibrosis of the pancreas and the teeth appear grey, brown or black. Another study conducted by Zegarelli, Kutscher and Fahn<sup>131</sup> suggested that there is striking relationship between tooth discolouration and the severity of fibrosis of the pancreas. They observed that tetracycline, given during the neonatal period, stained the primary teeth which appear yellow on eruption. As a group, tetracycline localised and deposited in tissues undergoing calcification at the time of administration. Davies and coworkers<sup>29</sup> reported upon post-mortem material and on two children with yellow teeth which fluoresced under ultraviolet light. One premature infant who died at 11 days of age, had been given a 5 day course of tetracycline, the last dose being given 48 hours before death. Both bone and teeth showed fluorescence, but it was more intense in the teeth, especially the dentine.

Wallman and Hilton carried out studies in 1961 and 1962.<sup>111,112</sup> In the 1961 study, they surveyed 64 full term children who had been given tetracycline during their neonatal period. Fifty of these babies were followed up and 46 were found to have yellow or brown discolouration of the teeth with or without hypoplasia. Early in the investigation, a pattern of tooth involvement which depended to some extent on the gestational age, emerged. In most full-term

babies, the pigmentation affected the gingival portion of the incisors and the incisal and occlusal portion of the canines and molars, respectively. They suggested that tetracycline does seem to pigment teeth when given in neonatal period. The greater the total dose of tetracycline per kilogram of body weight, the greater the chance of discolouration. The colour varies according to the age of the child; bright yellow pigmentation in younger children and brownish in older ones. In the case of a more premature group of babies, the yellow colour involved a greater part of the incisor tooth crowns. Enamel hypoplasia was found in the severely pigmented teeth.

Twenty one children who had been given oxytetracycline during their neonatal period were the subject of the October studies of Wallman and Hilton. They followed up records of 46 premature children using the birthweight of less than 2.5 kg. as criterion for prematurity. In the 21 children given oxytetracycline during the neonatal period, most of them given the drug in the first week of life, it was found that oxytetracycline does not seem to cause pigmentation or enamel hypoplasia. In their follow up of 46 prematurely born children, abnormal teeth were found only on the children given tetracycline. This is in spite of the large doses of tetracycline (210 mg/kg/birthweight). They likewise discovered that the dose of the drug is more important than the duration of treatment. They split longitudinally an extracted pigmented tooth and exposed one half to light and the other half in the dark. The

half that was exposed to light changed colour from yellow to brown and did not fluoresce on exposure to ultraviolet light. The other half remained yellow. They concluded that pigmented teeth exposed to light undergo brown discolouration.

Harcourt, Johnson and Storey<sup>45</sup> reported microscopic studies of incorporation of tetracycline in teeth when administered in growing children. Both macroscopic and microscopic changes were observed in dentine. Macroscopically, the first primary molars showed hypoplasia with most of the enamel worn down exposing a considerable amount of dentine. The crowns of the teeth were bright yellow. Fluorescent microscopy under ultraviolet light imparts a golden yellow colour to areas of dentine stained with antibiotic. Normal enamel and dentine presents a faint overall yellow green autofluorescence under ultraviolet light. Under low magnification in visible light the most obvious feature was the presence of a number of faint yellow lines in the dentine towards the occlusal of the teeth following the incremental line pattern. In the crown, these lines run parallel to the dentino-enamel junction. The enamel was unstained. Under high magnification, the dentine showed large interglobular areas. These, however, had no relation to the yellow lines. Harcourt's group also observed the presence of large numbers of bright golden yellow bands in the dentine under ultraviolet light. These bands followed the incremental lines and differed widely in size, distance apart and density. The dentino-enamel junction area fluoresced golden yellow. No obvious fluorescence was demonstrable

in enamel. Microradiographic examination showed a typical pattern of dentine with radiolucent dentinal tubules outlined by a denser area. Large numbers of interglobular areas which varied in size and shape were distributed more or less randomly throughout the dentine. Their presence was associated with the areas of dentine not coloured with tetracycline antibiotics.

In the 1962 study of a group of children, Weyman and Forteus<sup>124</sup> found seven with grey-brown colouration. Three of these children had no history of fibrocystic disease of the pancreas. As infants, two had oestitis in the maxilla and one in the mandible. They suggested that such grey-brown colouration was a result of tetracycline administration rather than the illness for which the drug was used. Coincidence of tetracycline therapy with fluorescent lines in the teeth, was likewise demonstrated by the same investigators in 1963,<sup>125</sup> in the case of a boy who died at 3 years 2 months of bronchopneumonia secondary to a thrombocytopenic purpura of unknown etiology. The teeth and the bones were found to be yellow, at post mortem examination. Tetracycline (Tetracyn), was given in six courses as follows: (a) 9 to 23 weeks of age = 14 weeks duration; (b) 1 year 17 weeks to 1 year 18 weeks = 9 days duration; (c) 1 year 24 weeks to 1 year 25 weeks = 1 week duration; (d) 1 year 28 weeks to 1 year 44 weeks = 16 weeks duration; (e) 2 years 2 weeks to 2 years 7 weeks = 5 weeks duration; and (f) 2 years 38 weeks to 2 years 39 weeks = 9 days duration. A vivid yellow discolouration of the bones and parts of the teeth was

evident on usual examination. The bone surface was uniformly stained except for small localised area, but the crowns of the deciduous incisors were pigmented only at the cervical half. The incisal half of the same teeth were relatively normal. The deciduous canine showed a yellow band in the middle third of the crown. Bright gold fluorescence of the yellow area specimen before sectioning was observed under ultraviolet light. There was clear evidence of six fluorescent bands in the dentine of the developing permanent incisor. This pattern was also true for the primary canine, but not in permanent canine and primary incisors. This undoubtedly is due to the different periods of development of these teeth. This histological investigation made on post-mortem material confirmed that discolouration is due to the tetracycline being incorporated in the tooth at the time of administration. The discolouration may also be found in the cementum, but not in the enamel.

De Veber<sup>30</sup> reported the case of a ten year old girl who received demethylchlortetracycline (Declomycin), for a period of six months. The dosage was 150 mg. four times daily. She developed teeth with bluish-grey discolouration, with a brownish deposit at the junction of the incisal and middle third of the affected teeth. Beckelman and Gingold<sup>8</sup> reported the case of a five and one half year old girl with yellowish-brown tooth staining. She received tetracycline therapy at the age of two weeks in treatment for pneumonia. The teeth had erupted with the yellow-brown coloured

bands present at the cervical third of the central and lateral incisors, the incisal thirds of the cuspids and most of the crowns of the molars. In 1964 Pinborg<sup>91</sup> cited a case of a seven year old girl who had received chlortetracycline, a total of 57 (17) G, from the eighth day after birth to four years six months of age for fibrocystic disease of the pancreas. All her teeth were stained grey, but the colour was more prominent in the primary teeth. A ground section of a primary molar examined under ultraviolet light, revealed several fluorescent bands in the dentine corresponding to the periods when tetracycline was administered. Bullen<sup>19</sup> in 1962, undertook a study of school children in British Columbia, of six and seven years of age. Six of the 1,281 children had 'lemon' yellow coloured teeth. Two cases occurred in primary teeth only, and four in permanent teeth only. Medical histories revealed that five of these children had received tetracycline in 1956 and 1957 for treatment of various infections. The sixth received tetracycline for prophylactic reasons following premature birth. Douglas<sup>32</sup> observed brownish yellow teeth in eight infants apparently following long-term administration of tetracycline to the six mothers involved. Kutscher and coworkers<sup>61</sup> reported the case of a 3 year old boy with discoloured deciduous teeth whose mother received tetracycline during the later month of pregnancy. A routine daily dose of 250 mg. every six hours, for a total of 23 days duration and 20.750 gm. was given. The child did not receive tetracycline after birth either prophylactically or therapeutically. Aside from

having the usual childhood diseases, the boy had been well. There was no evidence of systematic disorder, nor history of local factors such as trauma to warrant the generalised discolouration observed. This report further supports the theory that the tetracycline given ante-partum passes the placental barrier and may deposit and discolour teeth developing at the time.

Adler<sup>1</sup> studied a 2 year old child with pronounced bright orange-yellow pigmentation which was most severe at the gingival margin. The mother was treated with large doses of tetracycline throughout pregnancy for actinomycosis of the right mandible and cervical region. Weisman<sup>122</sup> in 1964 reported a 6<sup>1</sup>/<sub>2</sub> year old boy with severe brownish yellow discolouration of the four secondary molars; the primary teeth being normal in colour and appearance. The patient received tetracycline therapy from one year to six years of age for upper respiratory disease and severe tonsillitis. From the age of one to four years he received a total of 35 days tetracycline treatment, and from four to six, 88 days treatment. This gives a total of 123 days of therapy. Witkop and Wolf<sup>128</sup> in their study of children aged two and one half to seven years old, reported yellow brown discolouration associated with tetracycline therapy. Doses varied from 250-500 mg. per day or from 20 to 75 mg. per kg. of body weight per day. All seventeen children were found to have yellow to brown staining. Bright yellow fluorescence was observed under ultraviolet light. When the yellow pigmented teeth were exposed to sunlight they turned brown. The pigment loses its ability to fluoresce. Swallow<sup>110</sup> observed yellow

staining on teeth of a 1 year old girl whose mother had taken tetracycline for sub-acute myoblastic leukemia during pregnancy. The mother was given 500 mg. daily of tetracycline in addition to other drugs in her fourth month of pregnancy. No tetracycline was administered to the affected child during the neonatal or subsequently. This girl when examined, had a partly erupted upper and lower central and upper lateral incisors. The teeth were deep yellow from the cervical to the incisal and exhibited the characteristic golden yellow fluorescence under ultraviolet light.

In 1965, Cuttita and associates<sup>28</sup> attempted in their studies to determine the cause of discolouration of the teeth. On the basis of their findings, they concluded that tetracycline administration in therapeutic doses and for sufficiently long periods will be deposited in those teeth forming at the time. This deposition is observed as tooth discolouration in both primary and secondary dentition. Of the 70 patients with cystic fibrosis, 48 had evidence of discolouration ranging from light grey, brown or yellow to a dark brown or yellow. Histological study of ground sections was made using white light and fluorescence microscopy techniques. Under white light, the incremental lines appear yellowish in colour and under ultraviolet light a fluorescence characteristic of tetracycline was observed.

Macauley and co-investigators<sup>67</sup> studied 908 children in Oneida, New York. These children had been followed from birth until 4 to 7 years of age. The study population was stable and consequently the patients were available for long term study. Accurate drug

histories were obtained from obstetric and paediatric records. All the children involved were examined annually by dentists and paediatricians. Tetracycline was administered in the recommended dosages based on weights of patients and for periods averaging 4 to 10 days. From the results of this study it appears that the staining of the dentine and enamel is caused by the deposition of tetracycline during tooth development. They found that demethylchlortetracycline was the analogue that gives the strongest and brightest fluorescence, and oxytetracycline, the least. They also concluded, that tetracycline administered after the fifth month of gestation, and up to 6 months of age, may be incorporated in the deciduous teeth and result in the staining of these teeth. Tetracycline administered after 6 months and until 6 years of age may be incorporated in the developing permanent teeth. After 6 years, the secondary teeth are 90 per cent mineralised and there will be no tetracycline incorporated in areas where it would be of aesthetic significance.

Ibsen, Urist and Sognaes<sup>50</sup> gave comparative staining properties of different tetracycline in use. They used equivalent doses of chlortetracycline, oxytetracycline and N-pyrrolidinomethyl-chlortetracycline to determine the degree of tooth discolouration caused by each derivative. Their observations showed that clinical doses of the tetracycline or demethylchlortetracycline had a greater potential to discolour developing teeth than oxytetracycline. Both oral and intramuscular administration gave analogous result even though the intensity of stain was less. The N-pyrrolidinomethylchlortetracycline gave a fairly

deep yellow stain, but when exposed to sunlight grew faint.

In 1965 Henon<sup>48A</sup> made a survey of 1,707 school children from five to eleven years of age. Sixty or 3.5 per cent of these children revealed tetracycline pigmentation and the prevalence decreased with advancing age. Stewart<sup>107</sup> reported that the change takes place slowly and the yellow stain may still be discernable four to five years later. He substantiated Henon's finding that the teeth which are pigmented yellow gradually become darker. They change to shades of brown or grey and, as they do, lost the property of fluorescence under ultraviolet light. The labial surface of the incisors are first to darken, while the molars remained yellow for a longer period. They suggested that the change is due to the degradation of tetracycline and is accelerated on exposure to light.

Macauley and Leistyna<sup>66</sup> cited cases of tetracycline administration during pregnancy resulting in the staining of the teeth of the offspring. The first patient was that of a two and one half year old female whose mother received 600 mg. of demethylchlor-tetracycline daily for 5 days during her eighth month of gestation. The teeth were yellow and fluoresced under ultraviolet light. Within a year they had turned to yellow-brown and fluorescence was diminished. The central and lateral incisors were the most severely affected, while the cuspids and first molars were only moderately affected. The second molars appeared normal except for the occlusal surfaces. In the second case the mother of 3 year old twins had received 600 mg. of demethylchlor-tetracycline daily for five days during the 26th week

of her pregnancy. In both children the incisors, cuspids and occlusal surfaces of molars were stained yellow brown. They gave characteristic golden yellow fluorescence when examined under ultraviolet light. The third case studied involved a 3 year old girl whose mother received 600 mg. of demethylchlortetracycline daily for 4 days during her 28th week of gestation. All teeth were pigmented yellow-brown. No fluorescence was observed under ultraviolet light except on occlusal surfaces of the molars.

Applebaum and coworkers<sup>5</sup> studied teeth either as a result of exfoliation, extraction or autopsy. This involved 32 children with cystic fibrosis of the pancreas. These studies were undertaken to determine the presence, distribution and nature of the peculiar discolouration observed in the teeth of these patients. In ground sections prepared from 27 teeth, yellow pigmentation was found in all but 4 teeth. The pigmentation was usually found in the dentine or occasionally in enamel, or both enamel and dentine. The zonal pattern of discolouration usually followed the incremental lines of growth and the interglobular spaces in dentine. The fluorescent pattern appeared to parallel the yellowish pattern observed under white light.

Toaff and Ravid<sup>113</sup> examined 94 children ranging from 3 to 6 years of age whose mothers had been treated with tetracycline or oxytetracycline during pregnancy. The duration of treatment averaged 15 days with an average daily dose of 1,000 mg. Staining of the teeth was observed in only 13 children. None of the 47 children

exposed to tetracycline in utero from the 15th to the 24th week of gestation had discoloured teeth. Of the 15 children exposed to the drug from the 25th to the 28th week, only one was affected. However, almost half of the 25 children exposed from the 29th week to term had discoloured teeth. As a result of their observation, they stated that tetracycline treatment should not be withheld during the 25th week, but during 29th week to term.

Kowalewska, Szotowa and Winiarska<sup>58</sup> re-examined 25 children between the ages of two to five years, 18 of whom received tetracycline and the rest oxytetracycline on an oral dose of 50 mg/kg of body weight, for 3 to 5 days during their neonatal period. They observed that 15 given tetracycline had yellow to brown staining but only one given oxytetracycline had the same pigmentation. They concluded that newborn with low renal excretion of tetracycline and wide distribution of this drug in high concentration in both bones and teeth are likely to exhibit staining of their primary teeth.

Kutscher and coworkers<sup>62</sup> suggested that long-wave length ultraviolet light (3660 Angstrom), is the most effective for the diagnosis of the presence of tetracycline in teeth. The fluorescence is more intense and more discernable than that procured by a short-wave length ultraviolet light of 2537 Angstrom. It has been established that tetracycline fluorescence can be excited by ultraviolet light of varying wave lengths. This was used by investigators to determine comparative efficiency through observable fluorescence of the presumably tetracycline-induced discolouration

of the teeth in patients with cystic fibrosis of the pancreas. Weyman<sup>127</sup> described a girl patient aged 13 years who received frequent courses of tetracycline for bronchiectasis. The teeth were severely stained and very unsightly. The ground section of the incisor was viewed under ultraviolet light and a vivid multiple band fluorescence characteristic of tetracycline was observed. The enamel showed little fluorescence, but the incremental lines fluoresced strongly. It was found that the stain was in the enamel and did not show through the dentine. She suggested the probability of a higher concentration of tetracycline in the enamel than is indicated by the amount of induced fluorescence. Stewart<sup>108</sup> referred to Lunin's findings that 80 per cent of the 300 teeth randomly chosen from teeth extracted in two university clinics from Maryland, U.S.A., contained tetracycline deposits. Stewart also undertook a survey of teeth extracted from patients aged 7 to 15 years and from 3 to 5 years old from schools and dental hospital clinics, respectively. This was from May 1966 to May 1967. He observed that the deposits were visible as yellow fluorescent bands in the dentine when the teeth were sectioned and viewed under ultraviolet light. He concluded that the number of tetracycline positive teeth decreased as the patient's age increased. He also observed that the highest prevalence was among the teeth of the younger children (i.e. those born during and since 1962). This was the year in which the capacity of tetracycline antibiotics to discolour the dentition was first recognised. He found more discolouration in the crown of primary

teeth than secondary teeth.

Brearley and associates (1968)<sup>16</sup> studied 1,168 children of pre-school and young school age. This was made for the purpose of determining the prevalence of discolouration and/or hypoplasia on children who were given tetracycline during the period of tooth formation. Three groups of children residing in the suburban areas of Melbourne, Victoria, were the subjects of this study. Group I consisted of 797 children of 2 to 7 years of age. These children attended the pre-school clinic of the Melbourne Dental Hospital. The Group II children numbering 261 with ages ranging from 2 to 5 years, attended the extramural clinic of the same hospital. Group III composed of 200 children aged 18 months to 6 years, attended the clinics of the Melbourne City Council infant welfare centres.

The teeth of all the children were examined under daylight supplemented by artificial light, when necessary. The teeth were classified as light yellow-grey, (including grey), bright yellow or grey-brown or darker. Examination of the teeth for tetracycline-induced fluorescence was carried out with a 3,400-3,700 Angstrom unit ultraviolet lamp. Medical histories were obtained by interrogating the parents who accompanied the children. The examiners took particular attention of illness and drug administration, both prenatally and postnatally. The medical histories, however, of the Group II children were not available. The severity of discolouration was scored by a "Tetracycline Severity Index" (the value of the total

score divided by the total number of patients examined), as shown in Table VII. The condition of the two most severely affected teeth was used as the basis for the assessment of clinical discolouration.

The result of this study revealed that 20.1 per cent of the total number of children examined exhibited discolouration. Both discolouration and hypoplasia accounted for 4.02 per cent. The characteristic tetracycline fluorescence was found in 84.8 per cent. The investigators were of the opinion that the current theory of colour alteration of tetracycline discoloured teeth upon exposure to sunlight, was not supported by their study. The "Tetracycline Severity Index" demonstrated that in relatively small groups of children with high proportion of tetracycline-affected dentitions, the severity of staining and hypoplasia also increased.

The value of ultraviolet fluorescence in the diagnosis of teeth stained with tetracycline was questioned by Brearley's group. They observed that not all teeth which fluoresced were discoloured, but believed that not all tetracycline-discoloured teeth fluoresced. This condition was also observed in the Tamworth, New South Wales survey of 1967 (Martin and Barnard).<sup>71</sup> In the study of Brearley, the degree and shade of discolouration has not been established definitely due to the absence of information concerning the particular tetracycline administered and the dosage used.

Brearley and Storey (1968)<sup>17</sup> made a study of 1,000 extracted deciduous molar teeth which they examined under visible light and ultraviolet light. Of these, 82 per cent showed yellow fluorescent

Table VII. Tetracycline Severity Index. (Brearley, Stragis and Storey)<sup>16</sup>

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Degree of Discoloration	Classification of Effect	Score	Comment
No discoloration	Normal range of color	0	Aesthetically acceptable
Questionable	Light cream, no darker than the permanent tooth	0	
Mild	Light yellow-grey	1	Aesthetically objectionable
Moderate	Yellow to bright yellow	2	
Severe	Grey-brown or darker may involve two-thirds or more of the tooth crown usually darkest to the gingival 3rd	3	Aesthetically and structurally objectionable
Hypoplasia plus discoloration	Tooth morphology is abnormal; the enamel and/or dentition may be pitted. Transverse striations prominent across anterior teeth; there may be complete absence of cuspal enamel, and the cusps consist of spikes of dentin. Tooth discoloration from yellow to brown or darker	4	
Tetracycline severity index =		$\frac{\text{Value of total score}}{\text{Total examined}}$	

bands in dentine, characteristic of tetracycline. While 30 per cent of the tooth crowns were found to be moderately or severely stained, not all fluoresced under ultraviolet light. When severely pigmented teeth were examined microscopically, tetracycline was located in the form of dark brown, weakly fluorescent bands at or near the dentino-enamel junction. These bands were partly responsible for the macroscopic discolouration of enamel, but on removal of stained dentine, some discolouration remained, particularly in the cervical enamel.

B. Hypoplasia:- The possibility of the disturbance of matrix formation has not yet been resolved and the role of tetracycline is still the subject of much controversy. It is hoped this discussion will assist in the correlation of tetracycline therapy and the occurrence of developmental anomalies.

Bevelander and coworkers<sup>11</sup> showed experimental evidence that tetracycline inhibits skeletal formation in Larval Sand Dollar.<sup>5</sup> Evidence also showed that the drug affected growth of both teeth and bones. It may also produce deformity and shortening of the bones when injected into the embryo of developing chick. Later, in 1961, Bevelander and associates<sup>12</sup> administered high levels of tetracycline (5 mg. daily) during tooth development in rats for five successive days. Besides demonstrating incorporation and retention of fluorophore, they found partial inhibition in the increments of enamel and dentine. Wallman<sup>116</sup> called attention to the fact that second primary molars were deformed and had extremely sharp cusps as well as yellow staining, as a result of tetracycline

administration. He had observed this phenomenon in other babies who had received tetracycline for as little as one week immediately after birth.

In 1962, Wallman and Hilton<sup>117</sup> suggested that tetracycline rather than the illness for which the drug was given was directly responsible for enamel hypoplasia. Mello<sup>73</sup> in 1961 cited Brotzman and Kitscher as having confirmed Bevelander's findings in connection with mild hypoplasia in primary teeth relative to tetracycline treatment. Miller and Forrester,<sup>76</sup> however, disputed this claim. While they believed that there is evidence to support tetracycline diffusion into the forming tooth, this does not justify the deduction that the drug produces hypoplasia. Prematurity, he reasoned, is more likely to be responsible both for the enamel hypoplasia and for the need for tetracycline therapy. This contention was supported by Weyman and Porteus<sup>125</sup> in their study of 37 children. They found disturbances in development, but they felt that though hypoplasia occurred, this could not be related to tetracycline administration. Corroborating the previous suggestion, Harcourt and associates<sup>45</sup> found that tetracycline administered to growing children, besides giving bright yellow fluorescence was associated with a typical globular pattern of calcification in the dentine and the cementum. Although disturbance of dentine mineralisation was demonstrated in the form of interglobular areas, this did not mean that they were produced by the tetracycline. They suggested that probably, these were localised manifestations of chronic systematic illness.

Harcourt (1963)<sup>46</sup> examined teeth either extracted or exfoliated because of dental caries from patients who had suffered from neonatal jaundice. He reported on the incorporation of tetracycline in human enamel of patients who were given large doses of tetracycline for a period of two weeks after birth. The teeth were found to be very hypoplastic and were yellow brown in colour. Harcourt<sup>47</sup> the same year reported that administration of the tetracycline during the first few weeks or months of life produced a yellow staining often associated with hypoplastic enamel. The tetracycline became incorporated in the dentine and cementum calcifying at the time of therapy. This could sometimes be detected in areas of hypoplasia within the enamel, but not in the fully calcified matrix. Storey (1963)<sup>109</sup> found in his experiments with rats that higher doses of tetracycline (200-250), mg/kg/day, resulted in some rats having hypoplastic areas in enamel of the incisor teeth. He claimed that in man, it had been observed that enamel hypoplasia may be induced by high dosage levels of tetracycline given before the completion of amelogenesis.

In 1964, Nylen and coworkers<sup>86</sup> implicated tetracyclines as agents capable of producing a high incidence of hypoplasia in teeth of children. They suggested that in addition, partial inhibition of mineralisation had been demonstrated in the incisor of enamel of rats injected experimentally with the drug. They stated that the primary effect of tetracycline seems to be one of ameloblastic impairment leading to the formation of hypoplastic enamel. In addition

the impaired cells probably allowed passage of the tetracycline since only the affected enamel showed deposition of tetracycline. They considered the mineralisation disturbance as a secondary effect as a result of the antibiotic in the matrix or a change in matrix itself, resulting from cellular disfunction. Their observations were made on patients who received tetracycline during tooth formation. Witkop and Wolf<sup>128</sup> also associated localised enamel hypoplasia with tetracycline therapy. In their studies covering the six month period of 17 children, aged 2½ to 7 years old, 15 had hypoplasia of enamel of primary teeth and 2, of the secondary teeth (given at 9 and 11 months, respectively). The affected parts were the incisal edges and occlusal surfaces of the first molars. This observed hypoplasia was localized to those teeth undergoing calcification coincident with tetracycline administration.

Johnson<sup>55</sup> conducted a series of four experiments designed to study the gross histologic effect of the four homologues of tetracycline on teeth and bones. These are the tetracycline (TC), chlortetracycline (CTC), demethylchlortetracycline (DMTC), and oxytetracycline (OTC). Four groups of adult male Wistar rats were given each a different tetracycline for 21 days at recommended oral therapeutic dosages by weight. Examination of the continuously erupting incisor in visible light did not show any hypoplasia or change in pigmentation. Under ultraviolet light, fluorescence was seen earlier and to a greater degree in DMTC, and CTC compared to TC and OTC. In the second experiment, adult female rats were fed

different homologues of tetracycline in therapeutic doses throughout the 21 days of pregnancy. The drugs had no effect on litter size, and fluorescence was detected on the pups' teeth and bones. The third study made use of lactating female rats, fed therapeutic levels of various tetracyclines for 21 days after birth. Sucklings were noted to exhibit fluorescence of teeth and bones. In the fourth experiment, 74 weanling rats from 7 litters were divided into two equal groups. The experimental group received 39 daily therapeutic doses of the tetracycline commencing at the age of 21 days. No significant difference in weight gain or femur growth at the end of the 39 days was observed in the two groups. An apparent topical as well as systemic deposition of the fluorophore was discovered in fluorescent microscopic examination.

Harcourt<sup>46</sup> disagreed with Johnson and claimed that administration of tetracycline drug during the first week or months of life produced yellow staining often associated with hypoplasia of the enamel. Beckelman and Gingold<sup>8</sup> found hypoplasia in their studies. They stated that this condition, due to tetracycline, does not occur if given the drug after the teeth are fully formed. Sognnaes and associates<sup>105</sup> using monkeys and rats found that: (a) in the absence of disease, therapeutic doses of tetracycline do not necessarily interfere with the calcification process; (b) there is diffuse uptake of tetracycline in enamel; and (c) that tetracycline is not necessarily a "fellow traveller" of calcium per se, but rather, may be related to crystal surface position of calcium or some unidentified organic

compounds of calcium. Hall<sup>44</sup> in 1964 stated that it was considered that hypoplasia was most likely produced by metabolic changes as a result of the disease for which the tetracycline was administered. Large doses, however, cannot be discounted as a possible cause of hypoplasia. In the same year, Kline and associates,<sup>57</sup> studying children whose mothers were given tetracycline as antiluetic treatment during pregnancy, showed transplacental effect of tetracycline in the teeth of the offspring and that six showed varying degrees of hypoplastic enamel.

Bennet and Law<sup>9</sup> confirmed the presence of tetracycline in enamel of dogs' teeth when tetracycline was given during the period of amelogenesis. They also confirmed the presence of tetracycline in dentine, and enamel hypoplasia was observed. Eger and associates were referred to by Mello<sup>73</sup> as having shown that tetracycline causes a disturbance in the mineralisation of the dentine of rats. Mello also referred to Soentgen's works as having proven that tetracyclines in therapeutic doses produced significant hypoplasia. These same investigators claimed that interference with normal calcification of dentine and enamel developing at the time of tetracycline therapy was evident.

In 1966 Antalowska and Kralove<sup>3</sup> demonstrated through histologic examination that therapeutic doses of tetracycline per os provoked disturbance in the calcification of the dentine in rat incisors. The appearance and localisation is similar to those seen following the administration of fluoride. Kowalewska and associates<sup>68</sup> also reported in their studies of two to five years old children that some of them showed hypoplasia of the tooth enamel.

## V. THE EFFECT OF TETRACYCLINE ON OTHER TISSUES

Two early reviews, Dowling in 1955<sup>13</sup> and Musselman in 1956,<sup>78</sup> concluded that tetracycline was widely distributed in the body and had no affinity for any special tissue other than a tendency to accumulate in the organs through which tetracycline was excreted. These two investigators mentioned the liver, the kidney and the intestine as the places where tetracycline will be likely to accumulate. In 1957, Rall and coworkers,<sup>94</sup> in studies on tumors in mice and humans, noted a bright yellow fluorescence persisting in most types of tumor tissues where tetracycline had been taken previously. This finding suggested that such drugs, due to their selective uptake, could have an important use in histological investigations and the diagnosis of cancer. Waisman and Boldt,<sup>115A</sup> in 1957, found that tetracycline, when added in small amounts to a tryptophane-deficient diet stimulates growth especially in males. This improved growth effect of the tetracycline-treated diet was up to the level, but not exceeding that achieved when tryptophane was present in the diet. They regarded this stimulating effect as probably due to alteration in normal gut flora favoring amino acid metabolism. As mentioned earlier, tetracycline was also found to inhibit growth.

Malek,<sup>68</sup> was also of the opinion that tetracycline is incorporated into some tissues undergoing regeneration, necrosis and inflammation. His conclusion was based on the result of artificially-induced tissue damage in animals. De Veber<sup>30</sup> reported the case of a 10 year old girl given demethylchlortetracycline therapy. He observed

an unusual combination of photosensitivity, loosening of the fingernails and discolouration of the nails and teeth. This reaction was considered to be a toxic reaction to long-term administration of this tetracycline derivative which was used to combat recurrent osteomyelitis of the left fibula. Sandlow, Allen and Necheles,<sup>97</sup> using gastric washings and taking advantage of the fluorescent property of tetracyclines, were able to detect gastric malignancy. Malek and Kole,<sup>68</sup> studied penetration of various tetracyclines in experimentally produced myocardial infection. They found accumulation and prolonged fixation of tetracycline in muscle fibre of the heart.

Myers and Jaffe,<sup>80</sup> in their investigation to determine how much tetracycline is bound by the skeleton and the possible effect of skeletal maturation on the amount of tetracycline binding in growing teeth, observed that a two-fold increase in dosage of the drug in young animals is accompanied by a threefold increase in bone content of the antibiotic. He further stated that in older rats, no increase was discernible. Harris<sup>48</sup> in 1965 reviewed some of the recent research on tetracycline and he emphasised the toxicity and the clinical application and made reference to the different organs affected:

A. Eye:- Myopia during exposure which may be due to (a) accommodative spasm; (b) stretching of sclera; (c) changing of refractive power of aqueous and vitreous humors; and (d) changes in refractive power of the lens caused by <sup>edema</sup> edema of ciliary body, changes in structure of lens, fluid inhibition of lens, salt retention and

anterior displacement of the lens.

B. Kidneys:- The anti-anabolic effect of tetracycline on kidneys could be the result of inhibition of riboflavin enzyme system necessary for the maintenance of, or synthesis of tissue protein. It is inferred that the proximal tubule could be the site of this action and it is thought that one or more degradation products of tetracycline, anhydrotetracycline and epianhydrotetracycline, is responsible for nephrotoxicity and metabolic side effects.

C. Liver:- Tetracycline administered to pregnant women intravenously for urinary infection, and with doses ranging from 1.0 to 6.0 Gm and with an average of 2.4 to 4.4 Gm per day, were associated with the production of jaundice. On the third day, patients died of hepatic insufficiency with the cause of death diagnosed as acute liver disease.

D. Fingernails and Skin:- Photosensitivity, nail discolouration, onycholysis...a symptomatic triad produced in this order observed among patients using tetracycline or oxytetracycline.

Finn<sup>38</sup> wrote that tetracyclines are readily absorbed from the gastrointestinal tract, and therefore, are effective orally. While tetracycline could be administered intravenously or intramuscularly this route is often avoided because of severe local irritation and pain. Regardless of the route of administration, the tetracyclines are removed from the plasma by the liver and the kidneys. Care therefore should be taken, if used at all, in patients with advanced hepatic or renal insufficiency. Such precautionary measures, if not

observed, may lead to rapid plasma accumulation of tetracyclines, thereby enhancing toxic effects. Finn also mentioned that allergic and hypersensitivity reactions including skin rashes and drug fever have been reported with tetracycline therapy. A reaction of the skin in individuals treated with Demethylchlortetracycline (Declomycin) known as a phototoxic reaction, when exposed to sunlight has been reported. Long term therapy with this antibiotic is known to produce certain changes in the peripheral blood vascular supply including prolonged coagulation time and thrombocytopenic purpura.

Because of its wide bacterial spectrum, tetracycline affects the gastrointestinal flora, and gastrointestinal irritation may ensue. This is commonly due to an overgrowth of certain organisms, e.g. Candida albicans, and resistant coliform bacteria. Occasionally, suppression of the gastrointestinal flora may permit superinfection by resistant strains of Staphylococcus aureus. These drugs may also produce a brown or black coating of the tongue, a hypertrophic glossitis, or moniliasis of the oral cavity.